UNIVERSITAT POLITÈCNICA DE CATALUNYA
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MASTER'S THESIS

**Material Science and Engineering / EEIGM**

# MESOPOROUS SILICA NANOPARTICLES COATINGS ON TITANIUM SUBSTRATES


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# Abstract

Bones can suffer from damages due to ageing or traumatisms like fractures. When they are too damaged or if the fracture is too important, bones often need an exterior help to recover: the implants. The ideal implant maintains bone parts together and could promote bone regeneration and osseointegration. However, after placing the implant into the body, bone healing and regeneration sometimes don't work through properly. Indeed, the current bone implants sometimes lead to failure especially with patients suffering from diabetes mellitus (DM). In fact, it has been shown that DM is linked to the phenomenon called oxidative stress. The latter induces an accumulation of reactive oxygen species (ROS) which can cause dysfunction of the bone regenerating cells, the osteoblasts.

Titanium and its alloys are a well-known for their performances as biomaterials. The idea to prevent the implantation failure is to develop new functionalized implants. These implants must act like drug-carrier and be able to release the appropriate amount of drug locally. Many studies proposed different coatings for titanium that can be loaded with active principle. Among such coatings, the mesoporous silica nanoparticles (MSN) may be an efficient drug-carrying coating for titanium implants. The other coating techniques that have been studied involved weak bonds. However, the ideal coating needs to be bound strongly enough to remain stable on the titanium surface. Thus, the coating technique is a crucial parameter to obtain the ideal MSN coating.

This project aimed to develop MSN coating on titanium substrate. The process chosen for such coating was based on the silanization reaction. Silanized titanium substrates would react with functionalized MSN. This kind of reaction would induce the covalent bonding that is required between the nanoparticles and the titanium substrate to provide the right mechanical strength for the coating.

In this study, titanium substrate silanization process has been reviewed to be enhanced. Different conditions and parameters were tested: the activation technique, the coating time, the MSN coating solution concentration and two different silanes. The first trials were at first realized with the 3-(Triethoxysilyl)propyl isocyanate (ICPTES) as the silane precursor. Then the reaction was studied with 3-(Triethoxysilyl)propyl isocyanate (TESPSA). For each silane precursors, three activation techniques were tried: UV activation, NaOH solution attack and dioxygen plasma. In addition, ultrasound device was used to check the coating stability of the samples.

Each sample was controlled using the scanning electron microscopy (SEM) to study MSN coating morphology on the titanium plates. The best coating was obtained with an overnight coating reaction using TESPSA (2%, v/v), the MSN solution concentration was 5 g/L and the titanium sample was activated with dioxygen plasma.

# Acknowledgments

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# Glossary

| | |
|---|---|
| **[MSN]** | Mesoporous Silica Nanoparticles Concentration |
| **APTES** | (3-aminopropyl)triethoxysilane |
| **CTAB** | Cetyltrimethylammonium bromide |
| **DM** | Diabetes Mellitus |
| **EISA** | Evaporation-Induced Self Assembly |
| **FTIR** | Fourier transformed Infra-Red |
| **ICPTES** | 3-(Triethoxysilyl) propyl isocyanate |
| **MSN** | Mesoporous Silica Nanoparticles |
| **ROS** | Reactive Oxygen Species |
| **SEM** | Scanning Electron Microscopy |
| **SOD** | Super Oxide Dismutase |
| **TEOS** | Tetraethoxysilane |
| **TESPSA** | 3-(Triethoxysilyl) propyl succinic |

# Index

# 1. State of the art

## 1.1. Bones architecture and properties

From a chemical point of view, bones are constituted of both inorganic and organic material. Mostly hydroxyapatite (HA), which is the inorganic part (69%) and organic proteins (22%) such as collagen and non-collagen structural proteins. Bones are daily exposed to important mechanical stresses. The inorganic material, inorganic salts made of calcium and phosphate (HA), forms a crystalline complex [$Ca_{10}(PO_4)6(OH)_2$] that provides the specific mechanical properties of the bone. Therefore, bones are rigid and hard organs that can support the weight of the whole body, protect other organs and show regenerating and self-healing properties. Thus, bone turns out to be an interesting strong and light weighted bioceramic [1]. They can have different shapes and functions and they are made of complex internal and external structures.

More specifically, the cortical bone which is dense, solid and surrounds the marrow space and the trabecular bone which is composed of a honeycomb-like network of trabecular plates and rods interspersed in the bone marrow compartment. The external surface of the cortical bone is surrounded by the periosteum, except at the joints which are covered of cartilage. The periosteum is a fibrous connective sheath that contains blood vessels, nerve fibres, osteoblasts and osteoclasts. This envelope provides nutriment to the bone and protect it.

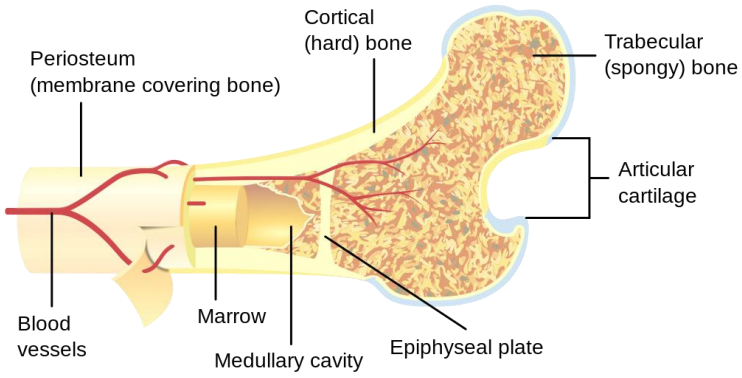



*Figure 1: Bone structure*[2]

Bones are evolving over time by different processes. They grow, reshape and remodel constantly to remain mechanically strong. The bone formation process is called osteogenesis or ossification. This process involves different components of the bone: the support cells osteoblasts and osteocytes, the remodelling cells called osteoclasts and the non-mineral matrix made of collagen and non-collagenous proteins (osteoid).

Osteoblasts are mononucleated cells derived from mesenchymal stem cells. Their role in bone formation is to form the bone matrix and regulate the activity of osteoclasts. As the matrix grows with the osteoid secretion, some of the osteoblast cells end up trapped in their own matrix. Then, they differentiate into osteocytes, which play the role of mechanical sensor cells inside the bone matrix. If the bone gets injured, the osteocytes will be able to send the information to the osteoclasts and the osteoblasts in order to resorb and reform the bone at the right place and the right time. [3]

Osteoclasts, as mentioned before, are the multinucleated cells in charge of the resorption of the bone. Indeed, these cells will degrade the bone matrix beforehand, so the osteoblasts can reform the matrix afterwards.

The two processes involved in bone formation (osteogenesis) are the intramembranous and the endochondral ossification. The former is a process based on a differentiation mechanism of mesenchymal stem cells which turn into osteoblasts, and the latter is characterized by the differentiation of mesenchymal cells into chondrocytes [4].

The ossification process follows three distinct steps: the synthesis of osteoid, the matrix mineralization and the remodelling of bone by resorption and reformation.

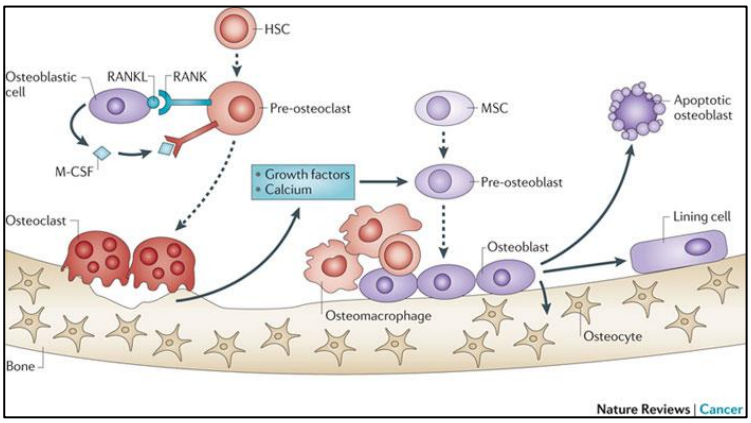



*Figure 2: Bone remodelling mechanisms*

These ossification steps are induced and controlled by three different mechanisms: osteoinduction, osteoconduction and osseointegration. Osteointegration is relating to the well integration of an implant. These processes are based on the action of proteins called growth factors and depend on the blood supply as well.

Osteoinduction is the basic biological mechanism involving the stem cells differentiation induced by various stimulations. The undifferentiated cells transform into pre-osteoblasts, then into osteoblasts and potentially into osteocytes. Later, Urist et al. showed that osteoinduction is influenced by the BMP growth factor released during trauma or bone-remodelling process. The term osteoconduction refers to the osteogenesis mechanism occurring on a surface stimulating the bone formation. Such surface is thus designated osteoconductive. Osteoconduction is strongly dependent on the previous osteoinduction. In the case of implants, the biomaterial used should be osteoconductive to assure bone growth. Finally, Osseointegration is a phenomenon which depends on both osteoinduction and osteoconduction.

Healing major injuries like fractures, or replacing bone parts requires implants to help bone reconstruction. However, placing an implant may turn out to be complicated and potentially dangerous for the patient. Indeed, this kind of complications are frequently observed when there is an abnormal amount of reactive oxygen species (ROS) in the organism. This is especially the case with patient suffering from diabetes mellitus (DM).

## 1.2. Diabetes mellitus and oxidative stress

*What is the diabetes mellitus?*

In simple terms, diabetes mellitus is a group of metabolic disorders that are the result of an insufficiency in production or action of insulin, a protein produced by the beta cells in the pancreas. The cells in the organism need insulin to react with special receptors allowing the glucose to enter and feed the cell. In most cases diabetes mellitus is diagnosed as type I diabetes mellitus (TIDM) or type II diabetes mellitus (TIIDM), respectively insulin dependent diabetes (insulin insufficiency) and non-insulin dependent diabetes (insulin secretory defect and insulin resistance).

The DM causes unusually elevated glucose levels in blood. The insufficient insulin ratio or the resistance to insulin results in reduced tissue uptake of glucose, inducing intracellular hypoglycaemia and extracellular hyperglycaemia. Long term elevation in blood glucose levels can cause various complications such as heart disease, blindness and kidney disease. Indeed, the glycosylation reaction occurs in such condition, leading to the formation of advanced glycation end products (AGEs) and eventually of ROS [5]. The diabetic pathogenesis can also be caused by other factors such as hyperlipidaemia and oxidative stress [6].

*What are the reactive oxygen species?*

Oxygen, in its dioxygen molecular form, is essential to all aerobic living organism. However, it may cause severe cell, DNA and lipid damages under specific conditions. In fact, by turning into an oxidant species the oxygen becomes a potentially dangerous component for the organism. The reactive oxygen species (ROS) is a term referring to a group of chemicals species which originally come from dioxygen in the organism. They are formed when the dioxygen is partially reduced, by electron transfer reaction. There are several forms of ROS, depending on the reduction degree of the dioxygen, they can have one or more unpaired electron.

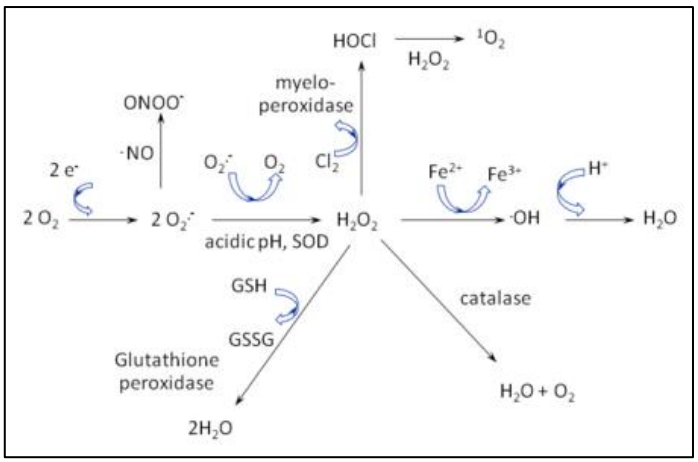



*Figure 3: transition reactions between ROS [2]*

ROS are highly reactive molecules; thus, they are likely to change from a species to another and to react with other biological molecules. ROS ensemble includes the superoxide anion $O_2^{\cdot-}$, the hydrogen peroxide $H_2O_2$ (which one is electrically neutral) and the hydroxyl radical $HO\cdot$ The superoxide anion and the hydroxyl radical are the most reactive species. The Figure 3 shows the different reactions leading to the formation of such species and also to their neutralization.

*How ROS, diabetes mellitus and oxidative stress are linked?*

ROS are playing different important roles in some physiological processes which are essential for the organism, such as intracellular signalling at atomic scale [7]. However, they also appear to be involved in many diseases and metabolic anomalies such as cancer, insulin resistance and diabetes mellitus.

These pathological states happen when the ROS concentration is either too low or more likely higher than the average level.[8] More specifically, the organism needs to stay into a homeostasis state. Indeed, an equilibrium between prooxidant and antioxidant species must be maintained in order to avoid oxidative stress issues. [9]

Oxidative stress as mentioned before occurs when the ROS concentration value pass over the threshold value. Diabetic conditions trigger some mechanisms which eventually induce the ROS production.

*How can Diabetes mellitus increase ROS production?*

Among them the mitochondrial electron transport chain (ETC) is the principal pathway to produce ROS, it produces initially the superoxide anion. However, they are also other processes producing ROS in diabetic conditions in addition of mitochondrial ETC. The figure 4 resumes the main mechanisms leading to ROS production.[10]

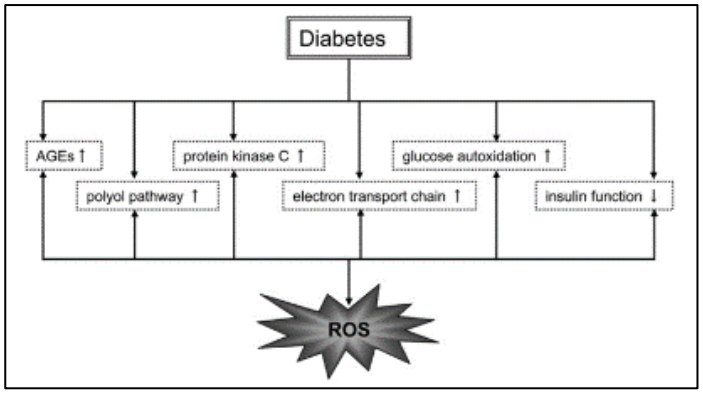



*Figure 4 : Mechanisms induced by diabetic conditions leading to ROS production*

The increased production of advanced glycation end-products (AGEs) is induced by high glucose concentration in blood. AGEs bind to their receptor and increase ROS formation by NADPH (nicotinamide adenine dinucleotide phosphate) oxidase which is a membrane-bound enzyme complex that faces the extracellular space.

In diabetic conditions, the amount of glucose is increased. Thus, more NADPH is needed to reduce glucose to sorbitol by the polyol pathway. This causes a decrease in glutathione formation and therefore, an increasing ROS concentration. In addition, the accumulation of sorbitol induces an important formation of fructose and nicotinamide adenine dinucleotide (NADH) which increases mitochondrial ETC.

Also, ROS can be formed by NADPH oxidase by the activation of protein kinase C isoform [11]. Finally, hydrogen peroxide may cause the glucose autoxidation in presence of $Fe^{2+}$ or $Cu^{2+}$ [12].

*How to fight against ROS?*

To prevent the ROS excess to harm the organism, enzymatic defences and antioxidant scavengers naturally help keeping the right ROS amount in the organism. The basic action of such defences is the reduction reaction of ROS, producing inoffensive species like water for instance.

Among these defences, the two types of SOD (superoxide dismutase) are the first defences against the superoxide anion, by turning it into $H_2O_2$. These types of SOD depend on the metal involved: the (Cu/ZnSOD) or SOD1, occurs in the cytosol, and the (MnSOD) or SOD2, occurs in the mitochondrial media. Small, neutral and rather stable, the H2O2 molecule can pass through the internal membrane of mitochondria and cause damages out of the mitochondria. Indeed, being outside the mitochondria the hydrogen peroxide can be reduced by metal-catalysed Fenton reaction to form the hydroxyl radical [6]. Then it is essential to neutralize the hydrogen peroxide by transforming it into water. This neutralization is allowed by the catalase mechanism. Moreover, when reduced, the glutathione can also decompose the hydrogen peroxide to water by peroxidase reaction. [13] The thioredoxins as well can degrade hydrogen peroxide. Antioxidants defences can also be species such as the vitamin C (water-soluble) or the vitamin E (liposoluble), which are exogenous molecules.

*What are the impacts of oxidative stress in bone disorders?*

The oxidative stress increasing concentration of ROS in diabetic conditions is thought to be a major cause to bone disorders such as osteoporosis (loss of bone mass)[10]. Indeed, overproduction and accumulation of ROS under diabetic conditions can lead to osteoblasts dysfunction and apoptosis [8].

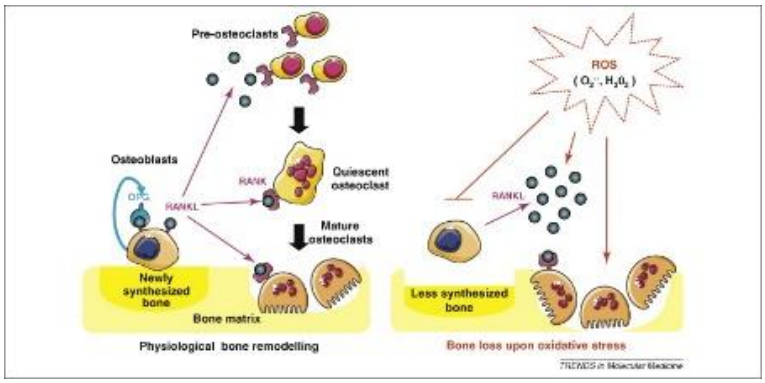



*Figure 5: ROS impact on bone remodelling*[47]

As seen on the previous figure, bone remodelling is carried out by osteoblasts and osteoclasts working in synergy. In a normal case, the pre-osteoclasts fuse by the binding of the ligand RANKL to its receptor RANK and form mature osteoclasts which resorb the bone matrix. This process is regulated by the secretion of the receptor OPG which links to RANKL and prevents the osteoclasts differentiation. However, in presence of ROS this regulation isn't efficient enough. The increased concentration of RANKL induces an increase in osteoclasts activity. Thus, the equilibrium between bone formation and bone resorption is not maintained. Therefore, the presence of ROS leads to a loss of bone mass. Also, age is a significant factor, since this phenomenon is frequently observed among the elderly. Thus, the bone remodelling

dysfunction is a real issue for the implant surgery because of the significant risk of failure. In addition, implant failures caused by oxidation have been observed and are thought to be induced by oxidative stress at endothelial cells level [14]. Other complications described as peri-implantitis [15], consists in the tissues inflammation around the implant. Peri-implantitis have been linked to the presence of ROS [16]. This is why the oxidative stress needs to be reduced, and the ROS activity neutralized locally.

## 1.3. Nifedipine and mesoporous silica nanoparticles

Titanium and its alloys are well-known biomaterial for their notable biocompatibility and mechanical properties similar to the bone ones. Titanium shows high specific strength and low elastic modulus; thus, they can provide the adequate mechanical support for the body. Moreover, an oxide layer is spontaneously covering titanium surface, preventing the corrosion [17].

However, the biological adhesion between bones and titanium isn't strong enough. Biological bonding between the implant and the bone could enhance the implant adhesion, by improving the osseointegration conditions. In addition, as ROS obstacles to osseointegration, implants would be much more efficient if they could cancel ROS activity by having an antioxidant function [8].

To achieve this objective, surface treatments of titanium and solutions to fight against the ROS in diabetic conditions have been studied. Various coatings such as chitosan [18], and tantalum [19] have been tested and showed improvements for osseointegration. Also, several antioxidants were studied in order to decrease the ROS impacts on bones. Curcumin for instance, has been studied as antioxidant treatment. Coupled with bone marrow transplant, the antioxidant action turned out to be efficient to reduce diabetes related damages [20]. The combination of coatings which promote bone healing and antioxidants treatment lead to the idea of a coating which could have both of these abilities.

This study is focalised on the utilisation of nifedipine as an active substance to cancel ROS activity. Nifedipine is calcium channel blocker which has been tested for its antioxidative action [21]. Indeed, nifedipine showed interesting results when it came to prevent lipid peroxidation. It also proved to be an efficient molecule against free radicals under its photodegraded form (nitrosonifedipine). Also, in a 2008 study, nifedipine turned out to reduce the superoxide anion production in type II diabetic mice brains [22]. The formula of nifedipine is represented below [23]:

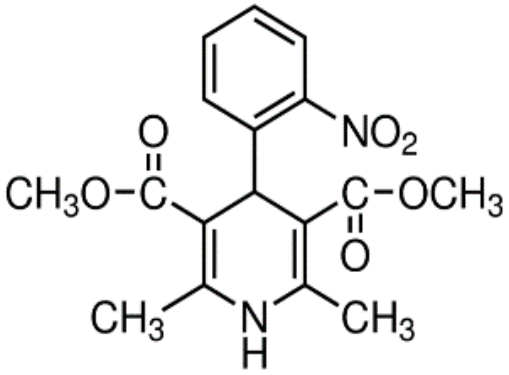



*Figure 6: Nifedipine molecule*

However, such drug has poor bioavailability. Indeed, this property is linked to the drug solubility in particular. As nifedipine shows a poor solubility in aqueous media, it needs a drug carrier to increase its efficiency in the organism[24]. Drug carriers must be able to be loaded and to release drug molecules in a controlled manner[25]. In the literature, several types of material have been studied. Mesoporous silica material proved to be advantageous materials for loading and release of insoluble drugs [26]. Indeed, they present a high surface area [27]. These types of structure are defined by the arrangement of pores which size is about a few nanometres, enough for a drug molecule to fit in [17]. These pores can be ordered in cubic, hexagonal or lamellar structure depending on the coating process conditions.
In the case of implants, drug-carriers need to be set at the surface of titanium to be able to release drug molecules locally. Therefore, mesoporous silica coatings have been developed to create innovative composite implants. In theory, such surface modifications can be realised following different coating processes. The main preparation technique of this type of coatings is the Sol-Gel process.

## 1.4. Coating techniques of titanium substrate

### 1.4.1. MSN and sol-gel process

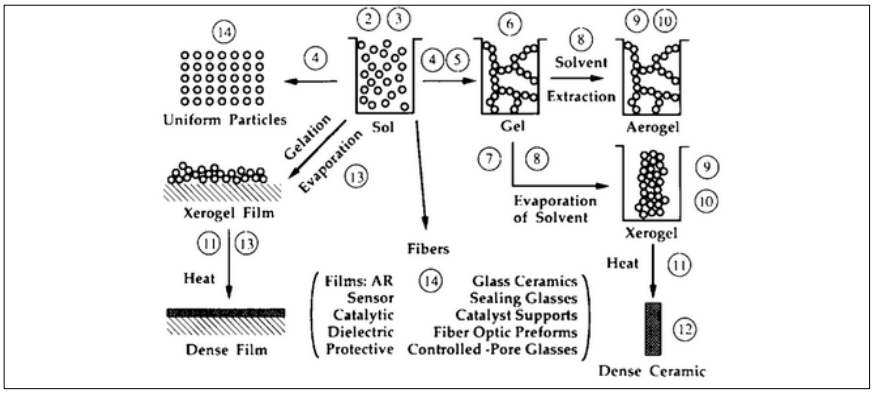



*Figure 7: Overview of the sol-gel process*

The sol-gel process has been considered as a useful technique to formulate nanoparticles, gel and mesoporous thin films until nowadays. Indeed, this process presents many advantages such as:

- better control of the chemical composition and microstructure (pore volume and size, surface area)
- homogeneous films in general
- reduction of the densification temperature
- simplicity, simple equipment, low cost

MSN as described previously, are synthetized by sol-gel processing. Many variations in the conditions of this process have been tested, since this process involves the use of different possible chemical product and solvent concentrations. To formulate MSN, the sol needs to be prepared with precursors, three types of compounds are required. Indeed, the MSN are formed in a media containing surfactants that will act like structure-directing agents, organosilane which is the base material to build MSN structure, and solvents such as water and ethanol. Acids or bases are also added to prevent the early hydrolysis and condensation of the inorganic

compounds. The concentration ratio between all these species must be set carefully, since it modifies the final type of mesostructure, as seen on the phase diagram below[28]:

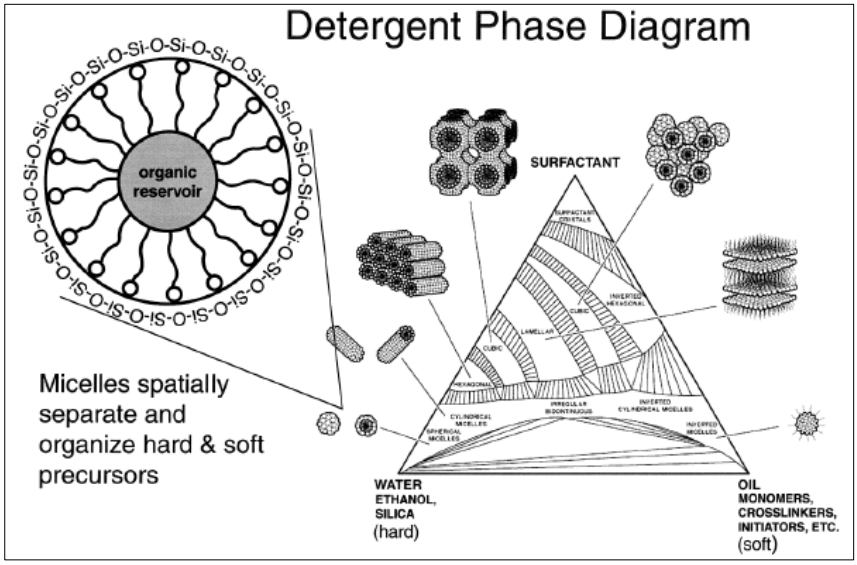



*Figure 8: Detergent phase diagram*

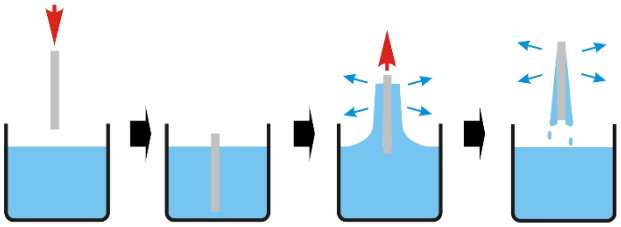
Once the solution is made, three reactions are occurring to form MSN: hydrolysis, alcohol condensation and water condensation. Thus, the sol is first containing colloidal suspension, and then the gel forms as the water and the alcohol are condensing. When the process is over, surfactant molecules must be removed by ionic exchange.

In this study, MSN are synthetized with two silanes: the TEOS and the APTES to functionalize the MSN surface with amine groups provided by the APTES. Such functionalization is obtained by co-condensation

The combination of coating processes and sol-gel techniques can lead to thin films [29]. This process is based on the Evaporation-Induced Self-Assembly (EISA) procedure [30].
Self-Assembly can be defined by the ability of molecules to build structures involving weak bonds such as Hydrogen bonds and Van der Waals forces. This phenomenon can be observed in presence of lipophilic and hydrophilic molecules or amphiphilic molecules (containing both parts lipo- and hydrophilic). Once these specific molecules (mostly surfactants and copolymers) in solution, if the critical micelle concentration is reached, they form micelles. Both spherical and cylindrical micelles can be formed depending on precursors ratio. As the solvent concentration decreases and the surfactant concentration increases by evaporation, the micelles self-assemble into hexagonal, cubic or lamellar structures [28].

### 1.4.2. Dip-coating process

The dip-coating process is an adequate process for complex-shaped samples. In this case dip-coating is set as a batch process, however continuous version of the process exists as well. This process follows five specific steps [31][32]:



Immersion → start-up → deposition → drainage → evaporation

*Figure 9: Dip coating process*

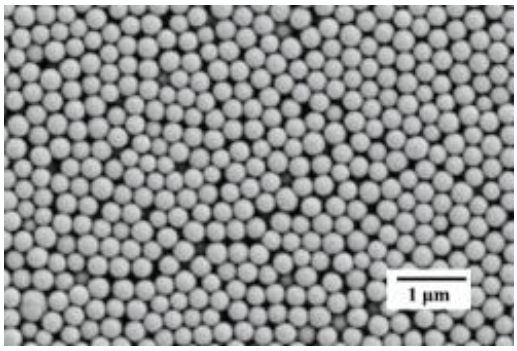



*Figure 10: SEM image of the self-assembly of polydisperse 296 nm silica spheres obtained by dip-coating*

First the sample to be treated is immersed into the precursor solution containing soluble silica and surfactant dissolved in water and ethanol. The micelle concentration is, at this point, below the critical micelle concentration. Then the dipping device starts the withdrawal of the sample, which drags the viscous solution. The withdrawal speed is maintained constant. As the sample move out of the solution reservoir, the solution flows down because of the gravitational draining. When the sample reaches 1 cm approximately above the sol level, the solvent starts to evaporate [33]. The ethanol evaporation induces the raise of surfactant concentration and therefore the nucleation of oriented mesostructure on the sample. As a result, thin mesoporous film is created. In fact, this is a result from the polycondensation and gelation reactions of the sol-gel process, occurring during the dip-coating process. The figure above shows a SEM image of a dip-coated sample [32].

### 1.4.3. Spin coating and electrochemical process

Spin-coating is a batch process designed to produce thin films using centrifugal forces to spread the coating solution on the sample to be coated. The fact that this process is very fast makes it attractive from the industrial point of view. The process is very simple, and the equipment needed isn't expensive. The substrate to be coated is rotating at velocity from 1000 to 10 000 rpm approximately. Catch-cup walls prevent the solvent to be released out of the machine. An aspiration system is also working to prevent the substrate to receive unwanted solvent droplets [34].

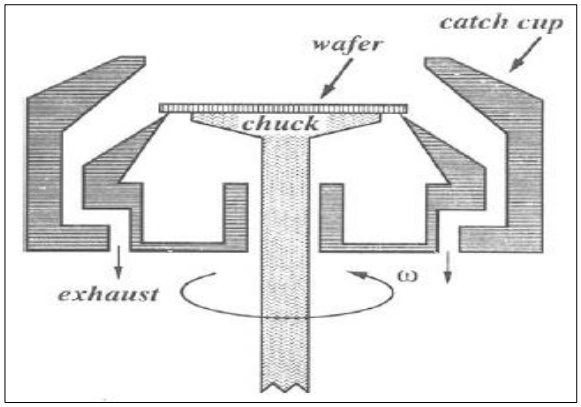



Figure 12: Schematic spin-coating device

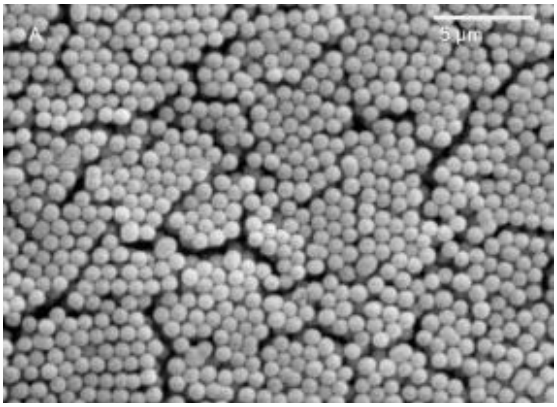



Figure 11: Spin coated sample

The first coating solution droplets are deposited on the sample rotating at low velocity (around 50 rpm). When the right amount of solution is added, the velocity increases up to 2000 - 4000 rpm during about 1 min. [35]

Also, the film thickness can be controlled by changing a spinning rate and a viscosity of precursor solution. The main weakness of this process is the shrinkage during the further steps (aging, drying) which is more significant.

The electrochemical process is known as called *electrophoretic deposition* (EPD). The nanoparticles are deposited on the substrate as a potential is applied between the two electrodes in the bath. This coating technique does not require expensive equipment and is quite simple to set up. Moreover, like dip-coating it allows to coat complex-shaped samples but with a good control of coating substrate. However, the following studies are about thick films formation ($10^2$ microns) [36].

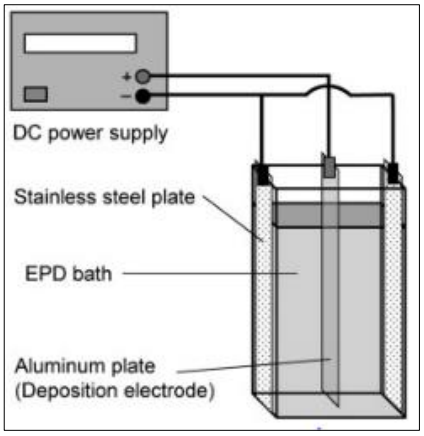



Figure 14: EDP process

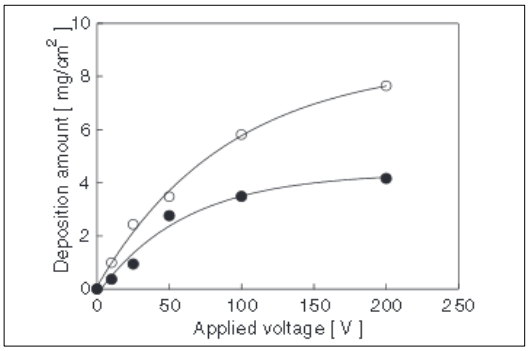



Figure 13: Effect of the concentration of particles in the bath

One major issue concerning these MSN coating on titanium is the bond weakness between the titanium surface and the coating [37]. Indeed, the only possible type of bonding between the substrate and the coating is weak interactions. Most of the studies are about sol-gel derived coating with nanoparticles. The covalent bonding of MSNs on Titanium would show an enhanced adhesion and better mechanical properties.

In this present work, the reaction between functional groups is studied as a way to attach nanoparticles to titanium substrates in a covalent manner. Indeed, activated titanium substrates could act like support for silanization. The organosilane used must have a functional group so the covalent bonding would be allowed by the reaction between the functionalized MSN and the functional group at the silanized titanium surface. Thus, the silanization represents a bright alternative compared to the other coating techniques.

In the present study, two different silanes were used, the covalent bond was either an urea bond or an amide bond, given that the reaction occurring during this process is involving either the isocyanate groups (ICPTES) or the succinic anhydride groups (TESPSA) and the amine groups at the MSNs surface [38], [39].

# 2. Objectives

The present work is the continuation of the previous project, namely, *Sintesis y Caracterisacion de Nanoparticulas de Silice Mesoporosa con un Agente Antioxidante Tipo Resveratrol, Florine.* The Main objective is the formation of a stable mesoporous silica nanoparticles coating able to carry and release nifedipine on titanium samples. The layer morphology, and the loading and release kinetic must be controlled.

To do so, the first step for this project is the optimisation of the silanization process. Afterwards the loading and release kinetics will be studied, and preliminary biocompatibility evaluated. To complete these objectives the following tasks have been realised:

1. Preparation of Mesoporous Silica Nanoparticles:

    - Synthesis of MSN using two precursors: TEOS for the structure and APTES to functionalize the surface. The aim of this step is to obtain nanoparticles with numerous pores and functional groups for further silanization.

    - MSN cleaning: removal of remaining structuring agent, CTAB, and drying of MSN.

2. Preparation of MSN coatings on flat titanium surface:

    - Polishing and washing of titanium plates in preparation for silanization. In this work, UV, NaOH etching and dioxygen plasma will be tested as activation technique for titanium plates.

    - Two different silanes will be studied for the silanization of titanium samples: TESPSA (3-(Triethoxysilyl) propyl succinic) and ICPTES (3-(Triethoxysilyl) propyl isocyanate). Then MSN coating will be studied using scanning electrons microscopy (SEM) and Fourier-transform Infrared spectroscopy (FTIR).

3. Loading and release study with nifedipine:

    - Drug loading protocol will be developed, based on the best results obtained in a parallel study (drug loading of MSN isolated). *(Effect of loading and release factors in vitro of MSN as drug carrier for nifedipine, Lorène Galmiche, 2018)*

    - Release kinetic of nifedipine will be studied. The release data will be obtained from UV absorption spectroscopy.

# 3. Materials and Methods

## 3.1. Ti sample preparation

In order to prepare flat titanium sample a polishing procedure was established. The objective here was to obtain a flat titanium surface without scratches or any defects. Titanium bar was obtained from c.p. grade 2 titanium provided by TECHNALLOY. The bar was cut into disks that were 3 mm thick and 10 mm in diameter. Mirror-like, smooth surfaces (Ra ≤ 40 nm) were achieved by grinding with SiC papers of a decreasing grit size (from P800 to P2400—European P-grade standard), followed by polishing with suspensions of alumina particles (6 and 1 lm particle size) on cotton cloths.

### 3.1.1. Bakelite Embedment

First and foremost, the titanium plates were placed into Bakelite polymeric resin. Each cylinder can carry five titanium samples. The Bakelite resin was used as a support for the titanium sample during the polishing. The machine used was the Struers Labopress-3.

The device heats the resin powder at 180°C and maintains a pressing force of 20 kN during 5 min. Then, the Bakelite cools down for 5 min, the pressure is maintained.


*Figure 15: embedment device*

### 3.1.2. Polishing

The polishing procedure was set in four preliminary cycles. These first steps aimed to obtain samples without major defects such as important scratches and planes.

*Figure 16: One plane and some scratches*

These are the following preliminary polishing cycles:

*Table 1: Polishing parameters*

| Time | Paper size | Force | RPM | Water |
|---|---|---|---|---|
| 10 min | 600P | 10 N | 150 | yes |
| 10 min | 800P | 10 N | 150 | yes |
| 10 min | 1200P | 10 N | 150 | yes |
| 10 min | 2400P | 10 N | 150 | yes |

The polishing machine used was the Struers Rotopol-31 working in the automatic mode: the device was polishing six Bakelite cylinders (30 titanium samples) at the same time. The force was applied by pistons.

After each cycle, every Bakelite piece was checked if there were any scratches or planes, in which case the cycle must be done again until no defect was observed. As the sample defects were often difficult to observe, the binocular microscope helped to get a better sample view. In addition, the papers must be replaced for each cycle. Indeed, black dots can appear and confirm that the paper is damaged. Once the five previous cycles completed, the samples needed to be polished more finely using polishing plate with alumina powder. The purpose here was to get a mirror-like surface.

First of all, the polishing plate must be wet by spilling deionised water. Then the machine was turned on and the alumina solution was added dropwise and carefully with deionised water during approximately one hour. Too much alumina would cause scratches. The samples must be checked from time to time to ensure that no scratch was appearing. A 1 micron alumina solution was employed to begin, then the plate was changed and the 0.05 micron alumina solution was added for another hour. In case of important scratches or planes, the sample concerned needed to be polished again with the previous step. When the samples surfaces were polished enough, the plates were removed carefully using a saw, a hammer and a small chisel. The Bakelite was caught in a vice, a cut was done near the surface with the saw, and then the Bakelite was broken using the hammer and the chisel.

### 3.1.3. Cleaning process

Once the polishing process was completed, the titanium samples are washed following different steps:

- Rinsing with acetone
- Sonication cyclohexane for 5min (three times)
- Rinsing with acetone
- Sonication $H_2O$ for 5min (three times)
- Sonication ethanol for 5min (three times)
- Sonication acetone for 5min (three times)
- Drying with $N_2$ or compressed air

These steps were necessary to remove all the organic residues from the titanium polished surface. The polished and washed titanium plates were placed in a 6x4 well-plate sealed with paraffin film. Once washed, the titanium plates were ready for the silanization.

## 3.2. Mesoporous silica nanoparticles synthetization

### 3.2.1. MSN synthesis protocol

The MSN synthesis protocol was taken from the previous project (Florine Bodet-Dubin, 2017).

The MSN produced for the study were made of two different silanes. Indeed, the two precursors were:

- The tetraethoxysilane (TEOS)
- The (3-aminopropyl)triethoxysilane (APTES)

The TEOS was used as the structure for the MSN, and the APTES was used to functionalize the MSN, as it has an amine functional group. Thus, amine groups of APTES would cover all the MSN surface and the inside of the pores. The reaction media was deionized water, with NaOH as base, and the structure directing agent was the cetyltrimethylammonium bromide (CTAB).

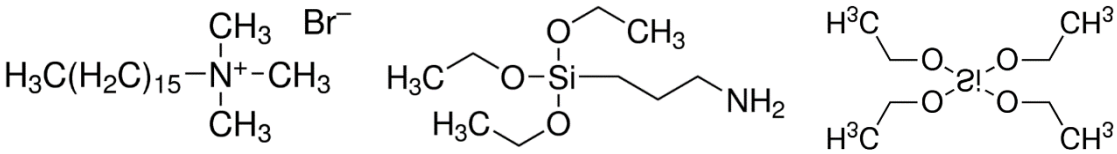


*Figure 17: CTAB; APTES; TEOS* [23]

First, the media was prepared in a 1 L bottle with 980 mL of deionized water and 2 g of CTAB. The mixture was heated to 80°C with magnetic stirring. Then 7 mL of NaOH were introduced. The precursor solution was prepared separately, 9 mL of TEOS and 2 mL from the APTES solution were needed. The functionalized amine groups concentration is easily set modifying APTES volume in the precursor solution. TEOS was added first because it is more stable, and it prevents the APTES from the hydrolysis. Then the precursor solution was added dropwise in the media. The solution must become white after the introduction of the precursors. The final solution was left at 80°C with magnetic stirring to form a vortex, which was important for the synthesis. The solution is then left for a week to let the MSN decant.

### 3.2.2. MSN cleaning protocol

Fresh MSN produced must be cleaned to remove the surfactant (CTAB) of the structure in order to have only the MSN functionalized with APTES. Indeed, CTAB is a dangerous compound for human organism, and needs to be extracted. Some studies reported methods such as calcination, however it wasn't used to preserve the amine groups at the MSN surface.

To clean the MSN, they were first centrifugated. MSN samples were placed into centrifugation tubes with solvent and were centrifugated for 10 min at 4000 rpm. This method used first water as solvent and then ethanol. After each centrifugation cycle, the solvent was changed. After the centrifugation step the MSN were filtrated using a Büchner funnel with a microfilter, connected to the vacuum pump. Then, 1.5 g of filtrated MSN were recovered and cleaned in a

plastic bottle for 24 h in 150 mL of methanol and 1.5 g of dissolved ammonium nitrate. The cleaning solution was stirred and heated to 60°C.

The ammonium nitrate is an extraction solvent. This compound is required as MSN and CTAB micelles are strongly linked by electrostatic interactions. It has been proved that the combination between alcoholic solution and ammonium nitrate is an efficient method to remove surfactant micelles. An ion exchange is occurring between the surfactant and the $NH_4^+$ ion. Later $NH_4^+$ ion is decomposing $NH_3$ and forming silanol groups on MSN surface [40]. This process was repeated three times. After the cleaning, the samples were dried in an oven at 60°C for 24 h.

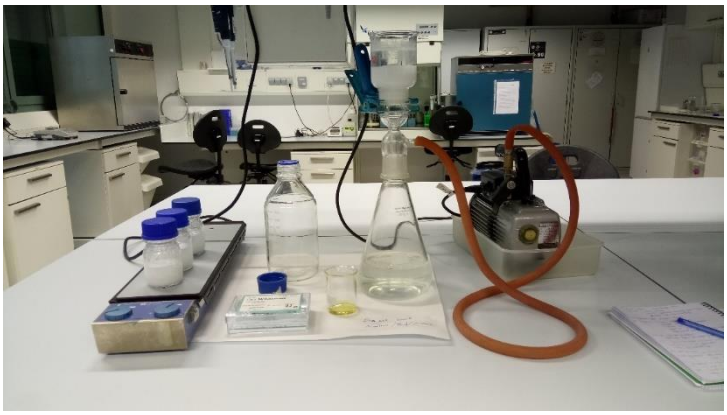


*Figure 18: Filtration and cleaning units*

## 3.3. Mesoporous silica nanoparticles coating on titanium process

### 3.3.1. Surface activation

Activation process is a crucial step for the silanization. It allows to modify surfaces by attachment or adsorption of functional groups to tailor surface properties for specific applications. In particular, it removes organic contaminants and it promotes surface oxidation and hydroxylation (OH groups) increasing its surface wettability. Surface modification was carried out by means of three different processes:

-*Oxygen plasma technique (plasma)*

It promotes surface oxidation and hydroxylation (OH groups) increasing its surface wettability. Plasma activated species include atoms, molecules, ions, electrons, free radicals, metastables, and photons in the short wave ultraviolet (vacuum UV, or VUV for short) range. Surface cleaning and activation were carried out by means of dioxygen plasma cleaning (Plasma Cleaner, Sterilizer PDC-002, Harrick Scientific Corporation, USA). After purging with 99.5 % pure oxygen and high vacuuming the samples were exposed to low electromagnetic radiofrequency radiation (between 8 and 12 MHz) for 10 min.

*-Sodium hydroxide treatment (NaOH)*

The titanium disks were immersed in 5 M of previously prepared sodium hydroxide in closed polypropylene flasks. They were placed into a furnace at 60 °C for 24 h. Afterwards the samples were cleaned and immersed in deionized water for 30 min twice, then rinsed with acetone and dried with $N_2$.

*-Ultraviolet lamps*

The titanium samples were placed underneath an UV lamp for 15 minutes to activate the sample surface.

### 3.3.2. Silanization process

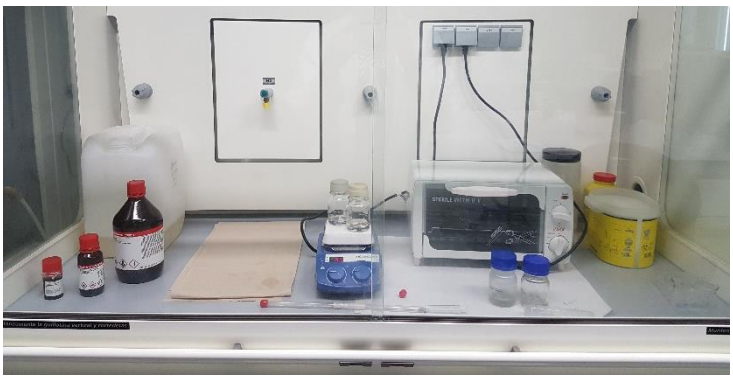


*Figure 19: Silanization set*

After the activation process, a silanization process was carried out. For this study, the organosilane used were the 3-(Triethoxysilyl)propyl isocyanate (ICPTES 95%, Sigma Aldrich), 3-(Triethoxysilyl)propylsuccinic anhydride (TESPSA 95% Sigma Aldrich). The silica precursor ICPTES has an isocyanate group, which is extremely sensitive to humidity. It needs to be handle very carefully.

First the titanium plates were activated as previously described. Meanwhile, the silanization reactor was prepared. The reaction needed to take place under dinitrogen atmosphere to prevent isocyanate groups, and succinic anhydride groups from hydrolysis reactions. Bottles of 150mL with elastomer septum were used to keep the reaction strictly under $N_2$ atmosphere. The bottle was carefully washed with distilled water, ethanol and acetone in order to eliminate impurities and any trace of humidity. Then the reactor was dried with $N_2$. The samples were placed in the bottle with a magnetic stirrer and the rubber top was placed.

To purge the air from the cleaned reactor, a needle connected to the vacuum pump was pricked in the elastomer septum. The air was vacuumed up for 5 min. Then the remaining air was purged of the bottle for 5 min using two needles, one for the $N_2$ flow input and the other for the output. The $N_2$ input needle was placed first, followed by the output needle in order to avoid any air introduction by air suction. This whole process needs to be carefully executed to prevent the titanium plates from turning over because of the $N_2$ flow (the polished side must remain up during the silanization).

Once the atmosphere conditions were set, 10 mL of toluene was added in the reactor, and the temperature was set to 70°C under smooth agitation. The reaction begins adding 0.3mL of DIEA (3% v/v, base) and 0.2mL (2% v/v) of the silane precursor (ICPTES or TESPSA) and goes on for 1 hour. The reaction occurring in the reactor are the following:

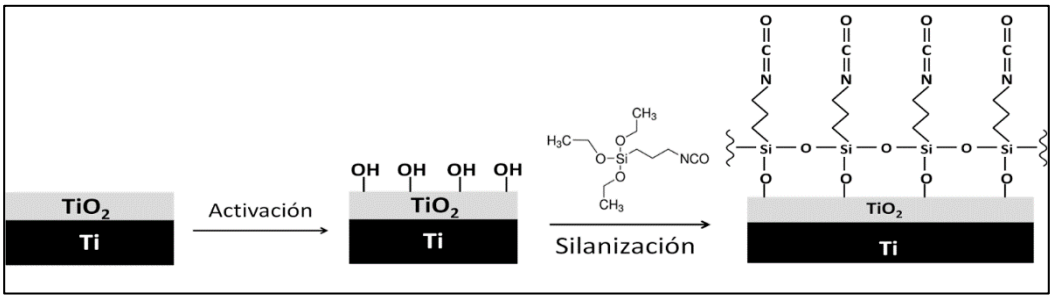



Figure 20: Silanization with ICPTES

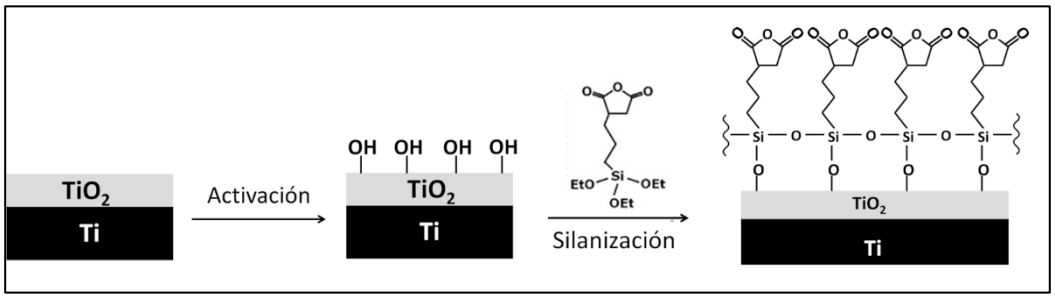



Figure 21: Silanization with TESPSA

### 3.3.3. Coating

After one hour the silanized plates were removed from the reactor and washed with toluene and acetone. The washing steps were the following:

- 3x2 min in anhydrous toluene (sonicating)
- 3 times rinsing in acetone

The washing solvent was changed each time. Then the plates were dried with $N_2$. This solution was simply prepared using 20 mL anhydrous toluene and samples of MSN. As the MSN could be agglomerated, the coating solution was sonicated before and after the introduction of the silanized plates to scatter the nanoparticles. Different MSN concentrations were tested through the experiments, to optimize the coating reaction and obtain a thin MSN layer.

Table 2 : MSN mass and corresponding concentrations

| **MSN mass** | | |
|---|---|---|
| 0.05 g | 0.1 g | 0.2 g |
| **[MSN] (MSN mass/20 mL anhydrous toluene)** | | |
| 2.5 g/L | 5 g/L | 10 g/L |

The MSN solution bottle with silanized samples was then put under agitation with an orbital stirrer. Different coating time were tested in order to obtain a thin and homogeneous layer of nanoparticles on the titanium oxide surface.

*Table 3: Coating times*

| **Coating time** | | | |
|---|---|---|---|
| 2 h | 6 h | ~12 h (overnight) | 24 h |

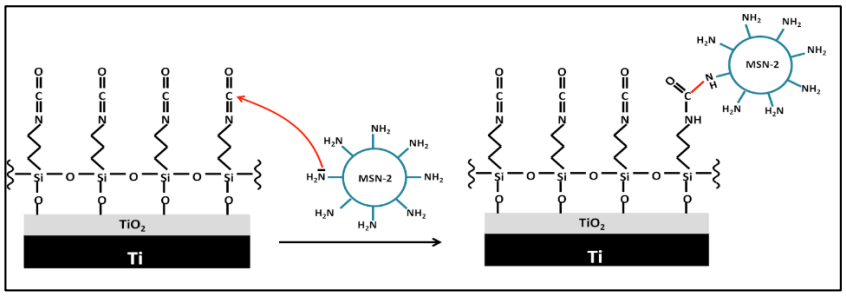



*Figure 22: Urea bonds formation between the ICPTES and the MSN*

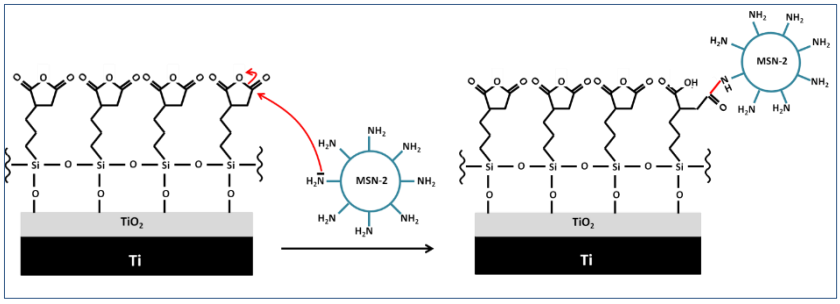



*Figure 23: amide bonds formation between the TESPSA and the MSN*

During the coating step, the amine groups on the surface of the MSN are reacting with the isocyanate groups or the succinic anhydrides to form respectively urea bonds or amide bonds. These covalent bonds are required for the coating mechanical strength.

After the coating step, different washing process were tested:

- using the orbital stirrer (3x2 min with toluene and acetone)
- using the ultrasound device (3x2 min with toluene and acetone)

Then the coated titanium samples were then dried with $N_2$.

## 3.4. Characterization techniques

### 3.4.1. SEM

In this study the scanning electron microscopy has been used to check if the titanium plate is well-covered by a mesoporous silica nanoparticles thin film. Two different SEM were used for the observations: the "desktop SEM" PhenomXL (Phenom-World B.V.) and the scanning electron microscope Zeiss Neon40 (Carl Zeiss NTS GmbH, Oberkochen, Germany). The PhenomXL was the most used.

Scanning electron microscopy is widely used to analyse the topography. The electron beam is generated by the electron gun. By applying an accelerating voltage, the electrons flow through a hole in the anode. Then, the electron beam goes through a series of magnetic lens that condense the beam and focus. There is also an aperture plate between the condensing lens and the objective lens which regulate the probe current. The beam reaches next the specimen placed on a stage which can move horizontally in the plane (x,y) and vertically (z axis). The observations are performed under vacuum in order to avoid any interaction between electrons and gas particles.

When the beam hit the sample surface various electromagnetic waves and electrons are emitted. The image we obtain while observing the sample in the microscope is due to the emitted electrons detection. More specifically the secondary electrons (SE) and the backscattered electrons (BE), detected respectively by the SE detector and the BE detector.

Figure 25: Zeiss Neon40

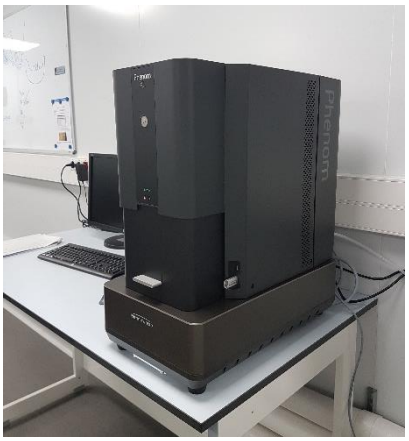


Figure 24: PhenomXL

First the sample were placed on special pins with carbon adhesive discs. Then, the assembly was coated with carbon by thermal evaporation under vacuum. The pin was then placed on the sample stage. The height of the sample top was set using a thumb wheel. Indeed, to get the best image quality, the distance between the end of the SEM column and the sample must be near 2 mm, and should not exceed 6 mm. Such distance is called the working distance (WD). Once the stage was in the chamber, the sample can be observed at low vacuum using the backscattered electrons detector (BSD). However, the image quality is not very high. The optimal vacuum condition was achieved after 5 min. Such vacuum condition is required to switch to the secondary electrons detector (SED), which gives the best quality images. Moreover, the observations were realised using an acceleration voltage of 15 kV. All the sample surface was examined with attention for each sample.

### 3.4.2. FTIR

The Fourier-transform infrared spectroscopy characterization technique was used to verify the MSN functionalization with amine groups, and to confirm that the MSN were free of CTAB and ammonium nitrate.

The device used for the study was the FTIR Nicolet 6700, which uses a He/Ne Laser and takes measurements from 400 to 4000 $cm^{-1}$. Given that the samples studied were nanoparticles, it was necessary to analyse them in transmission mode. Thus, thin pellets made of KBr and MSN were formed with a press (solid dilution) and were analysed with the software OMNIC 8.

*Figure 26: Press*


*Figure 27: FTIR device in transmission mode*

This characterization technique is based on the interactions between electromagnetic wave and matter. Indeed, the material will absorb specific wavelengths as the beam hit the sample. This absorption is due to molecular vibrations. Indeed, when atoms absorb the wave energy, they vibrate in different modes (such as stretching, rocking, twisting etc.). It is then possible to identify bonding between different atoms by studying the absorption spectrum.

# 4. Results

MSN were synthetized following a specific sol-gel process. As seen on the further SEM images, the MSN size was about 100 nm as expected.

After the synthesis, the MSN were cleaned by centrifugation and filtration process. In comparison to the parallel study about the MSN synthesis, the centrifugation step to clean the MSN could be skipped, since the filtration was enough to clean MSN. Moreover, the filtration process is faster.

## 4.1. MSN synthesis

As the MSN had been synthetized, FTIR characterization was carried out to find out if the samples were acceptable and thus, able to be used for the further silanization experiments. The figure below shows the spectrum of CTAB (blue), cleaned MSN (purple), ammonium nitrate (green) and dirty MSN (red):

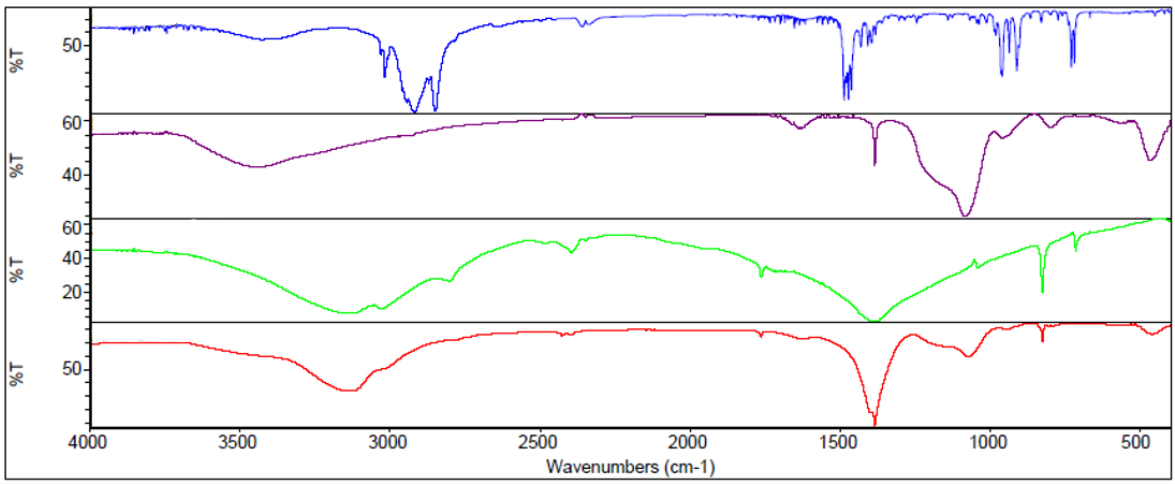



*Figure 28: FTIR results; CTAB (blue); cleaned MSN (purple); ammonium nitrate (green); dirty MSN (red)*

The table below resumes the matches between different peaks:

| **Wavenumber ($cm^{-1}$)** | |
|---|---|
| 3450 | Silanol (Si-OH) |
| 1630 | Amine ($R\text{-}NH_2$) |
| 1400 | Alkane (C-C) |
| 1000-1300 | Si-O-Si |
| 960 | Silanol (Si-OH) |
| 800 | Si-O-Si |
| 460 | Si-O-Si |

As seen on the spectrums, the ammonium nitrate (green curve) presents two major peaks located at 3150 $cm^{-1}$ and 1400 $cm^{-1}$. It appeared that the poorly purified samples (red curve) presents the same peaks as for the ammonium nitrate. Moreover, the dirty MSN peak at 3150 $cm^{-1}$ shifted from its initial position at 3450 $cm^{-1}$ (as seen on the purple curve). This can be

explained by the presence of ammonium nitrate in the dirty MSN. In addition, the dirty MSN signal peak at 1400 $cm^{-1}$ was much stronger than the one of the cleaned MSN. Finally, the peak in the range from 1000 $cm^{-1}$ to 1300 $cm^{-1}$ as seen for the cleaned MSN corresponding to the Si-O-Si bonds, drastically decreased on the dirty MSN curve. This meant that the ammonium nitrate was efficiently removed in the case of cleaned MSN. Regarding the blue curve, no correspondence has been found with the other curves, which means that the CTAB was efficiently removed.

## 4.2. Silanization

In a first phase, the conditions of the silanization were chosen according to the ones established in the previous project to keep consistency with the work done before. Several parameters of the silanization process were studied for the purpose of the optimization process. At the beginning these parameters were the type of silane, the coating solution concentration, and the coating time. However, some changes in the process such as the activation technique and the washing step after the coating step, were also tested.

### 4.2.1. ICPTES

#### UV Activation

The ICPTES was tried as silane precursor, as it was used for the previous work. The first experiments conditions were the following:

*Table 4: Experiment 1*

| **Silanization** | |
|---|---|
| Activation | UV (15 min) |
| Silane | ICPTES (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 10 g/L |
| Coating time | 24 h |
| Ultrasound | No |

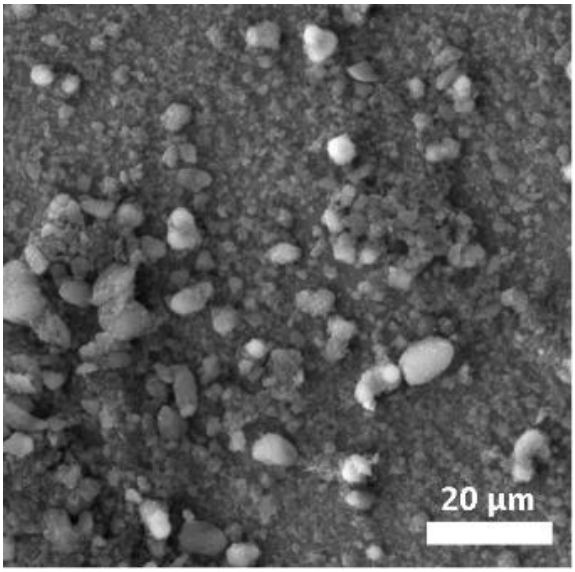



*Figure 30: experiment 1 ICPTES x3000*

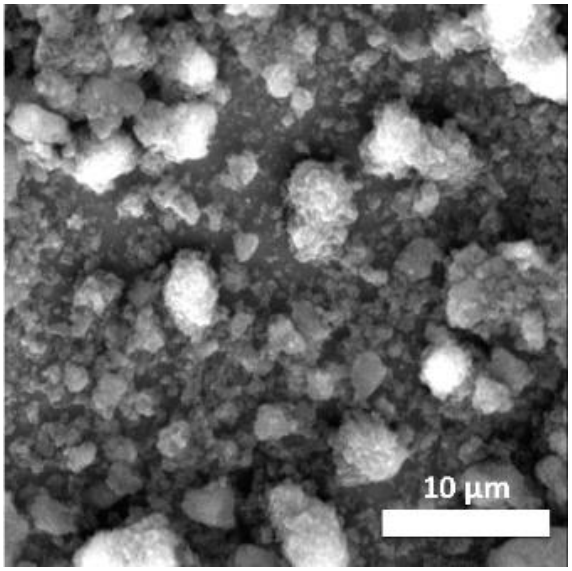



*Figure 29:experiment 1 ICPTES x8000*

The coating obtained was too thick and irregular because of the important amount of material. Indeed, as seen on the SEM images, the coating showed many clusters of nanoparticles. The size of these clusters reached until approximately a few tens of microns. The layer was thick enough to be visible without any magnifying glasses. The coating seemed to be attached with covalent urea bonds, but the numerous clusters gave the impression that the MSN could also be attached by weak interactions (physical adsorption). Therefore, it was impossible to tell whether the coating is covalently attached or not, and if it was, in which proportion relative to weak interactions.

To reduce the thickness of the layer, two strategies were applied: decrease the MSN concentration, decrease the coating time. The MSN concentration was decreased first. Thus, the coating conditions became:

*Table 5: Experiment 2*

| **Silanization** | |
|---|---|
| Activation | UV (15 min) |
| Silane | ICPTES (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 5 g/L |
| Coating time | 24 h |
| Ultrasound | No |

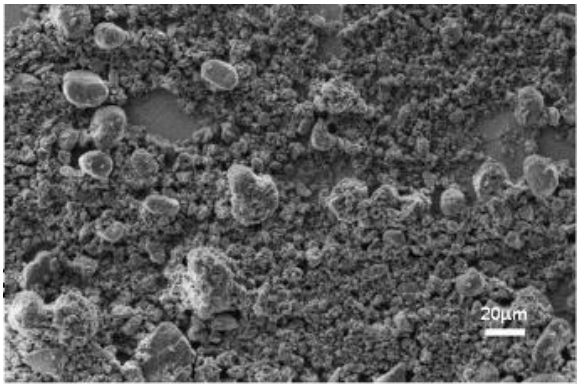



*Figure 32: experiment 2 ICPTES x1050*

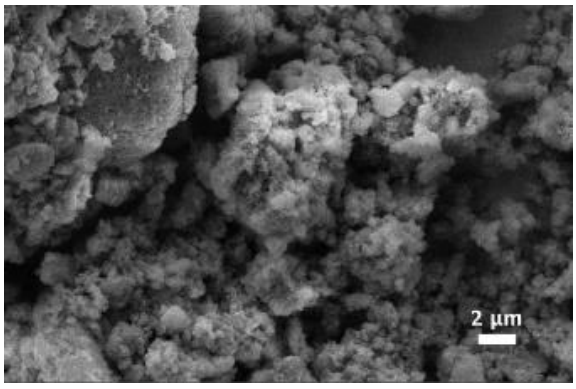



*Figure 31: experiment 2 ICPTES x9950*

As for the first experiment, a lot of clusters remained attached to the titanium silanized surface. Therefore, the coating time was decreased again to reduce the amount of material. Then the sample was immersed for 6 hours only.

Table 6: Experiment 3

| **Silanization** | |
|---|---|
| Activation | UV (15 min) |
| Silane | ICPTES (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 5 g/L |
| Coating time | 6 h |
| Ultrasound | No |

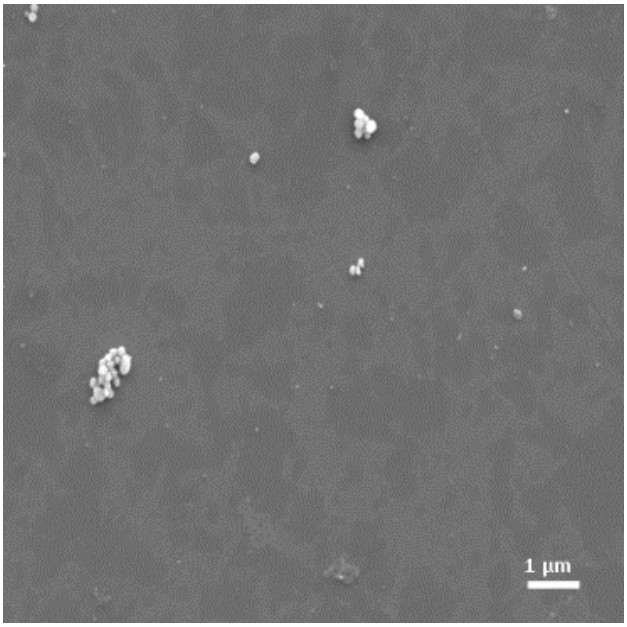



Figure 33: experiment 3 ICPTES x10000

It appeared clearly that the nanoparticles didn't bind the surface. This result was confusing because the amount of material was drastically reduced as if the reaction did not occur at all. As the problem was thought to come from the activation process, the process was changed for the next experiments.

## NaOH etching activation

The samples were immersed in a NaOH solution at 60°C for 24 hours. The coating time was set to approximately 12 hours (overnight experiment) with a 5 g/L MSN concentration to get more material on the surface. In addition, after the coating step, one plate was sonicated, and the other wasn't. This final step of ultrasound washing was established to check the coating stability. The result is shown in the figure below:

*Table 7: Experiment 4*

| **Silanization** | |
|---|---|
| Activation | NaOH (24 h, 60°C) |
| Silane | ICPTES (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 5 g/L |
| Coating time | Overnight |
| Ultrasound | No |

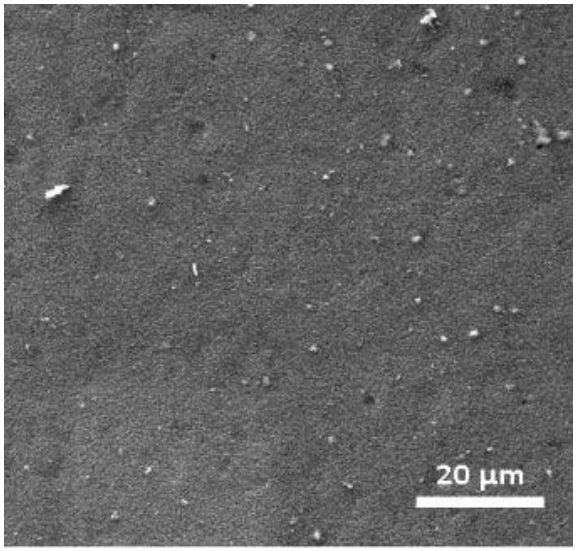



*Figure 35: experiment 4 ICPTES x3000*

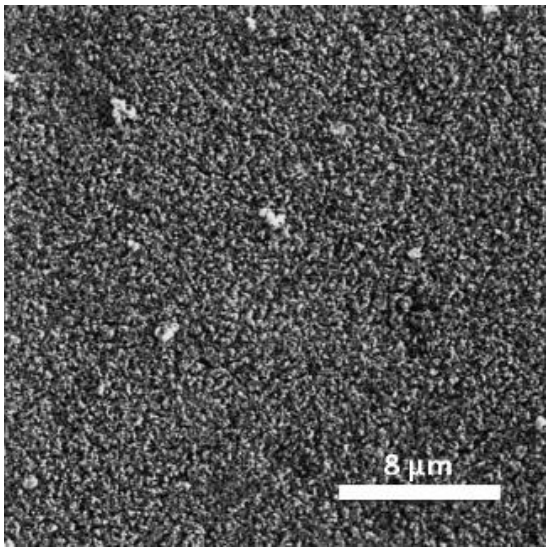



*Figure 34: experiment 4 ICPTES x10000*

Table 8: Experiment 5

| **Silanization** | |
|---|---|
| Activation | NaOH (24 h, 60°C) |
| Silane | ICPTES (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 5 g/L |
| Coating time | Overnight |
| Ultrasound | Yes |

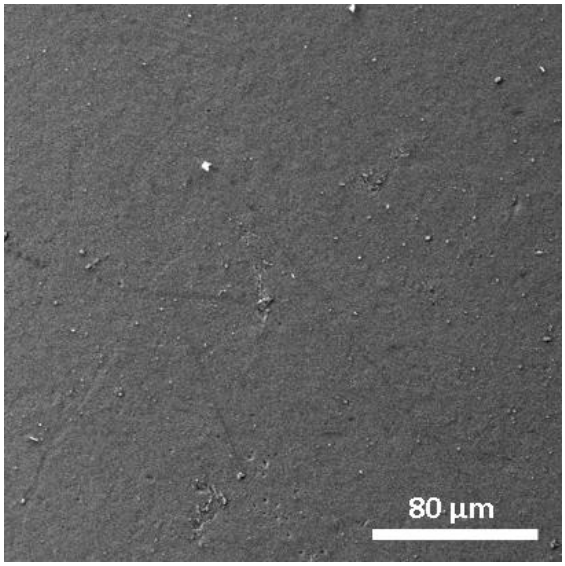



Figure 37: experiment 5 ICPTES x1000

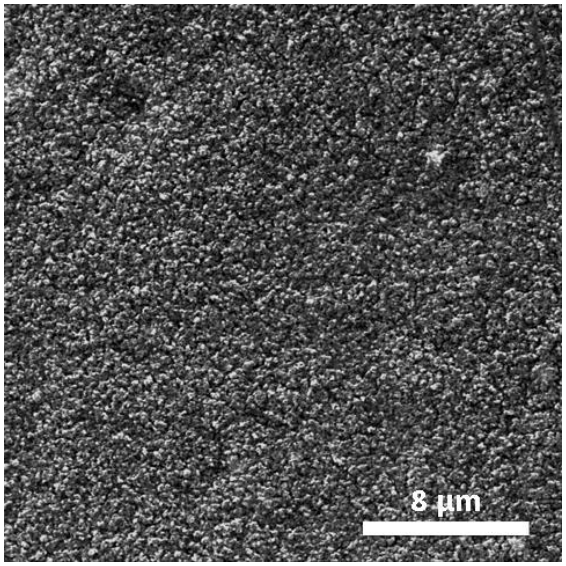



Figure 36: experiment 5 ICPTES x10000

The first thing that appears obviously is the change of the surface aspect. The titanium surface was no longer smooth. Indeed, the NaOH activation solution increased the surface roughness homogeneously. The MSN coating didn't attach properly, there were only a few clusters and nanoparticles disseminated on both sonicated and non-sonicated samples. However, there was slightly more material on the sample non-sonicated. Therefore, the sonication step seemed to remove clusters more efficiently. It seemed that the origin of the problem could be the ICPTES quality.

## Ninhydrin test

At this point of the experiments, a ninhydrin test was executed to determine if the ICPTES was degraded or not. Indeed, this test is used to detect the presence of amine groups. If the ICPTES was degraded by hydrolysis, the isocyanate groups would be transformed in amine groups. As seen on the picture below, the blue colour showed that the isocyanate was degraded.

Therefore, the efficiency of the silanization could be affected by this degradation. However, the change in the coating seemed to be too significant to be only caused by the ICPTES degradation. Moreover, the ninhydrin test is very sensitive, and could detect a slight degradation of ICPTES.

A change in the activation technique was firstly considered in order to solve this coating problem.

*Figure 38: Ninhydrin test tubes*

Finally, a last experiment was realised using a new ICPTES, and MSN recently synthetized. The conditions chosen for the silanization and the coating were rather similar to the first experiment, apart from the activation technique which was dioxygen plasma. To test the coating stability, ultrasound washing, and manual washing were done.

*Table 9: Experiment 6*

| **Silanization** | |
|---|---|
| Activation | $O_2$ Plasma (10 min) |
| Silane | ICPTES (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 5 g/L |
| Coating time | 24 h |
| Ultrasound | Yes |

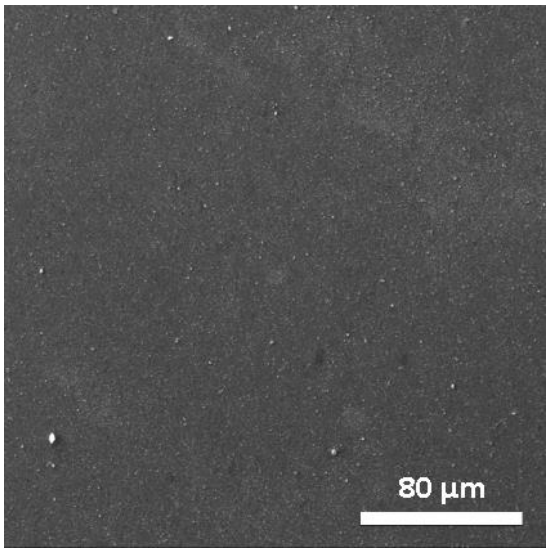



*Figure 40: experiment 6 ICPTES x1000*

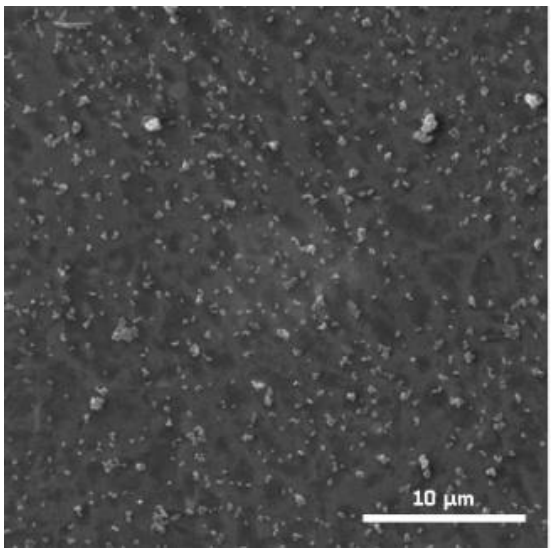



*Figure 39: experiment 6 ICPTES x8000*

### 4.2.2. TESPSA

#### UV Activation

In a second phase, TESPSA was used as organosilane to attach MSN with an amide bonding to the titanium.

In the first experiments, the titanium plates were activated using the UV activation technique. The first experiment conditions were the following:

*Table 10: Experiment 7*

| **Silanization** | |
|---|---|
| Activation | UV (15min) |
| Silane | TESPSA (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 5 g/L |
| Coating time | Overnight |
| Ultrasound | No |

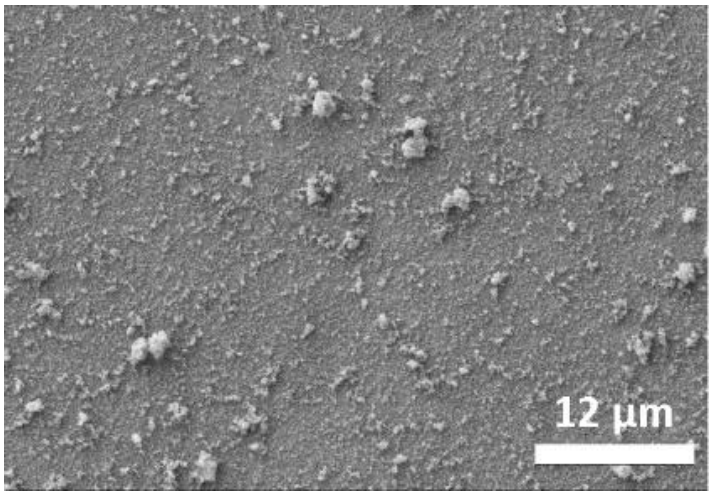



*Figure 41: experiment 7 TESPSA x4150*

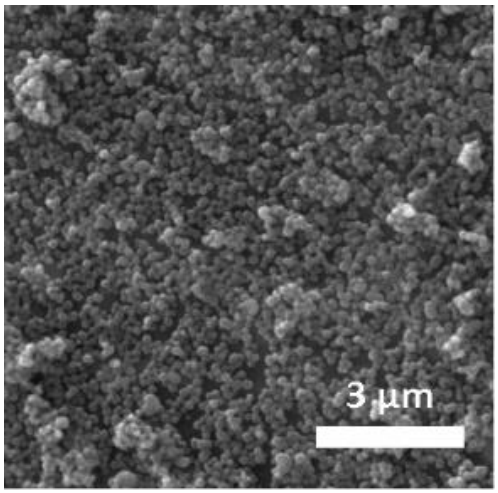



*Figure 42: experiment 7 TESPSA x27000*

As for the very first experiment with ICPTES, the SEM images showed an important amount of material on the whole sample surface. However, this layer wasn't homogenous, since MSN clusters were remaining. In view of this observation, two hypotheses regarding the kind of bonding could be considered: the layer seemed to be attached with covalent amide bonds, but the numerous clusters gave the impression that the MSN could also be attached by weak interactions (physical adsorption). Therefore, it was impossible to tell whether the coating is covalently attached or not, and if it was, in which proportion relative to weak interactions. MSN

coating is occurring under constant agitation (orbital shaker). To remove this excess of material which wasn't covalently attached, ultrasounds washing was applied after the coating for the next experiments.

To approach the right amount of material for the coating and remove remaining the clusters, two experiments were executed in parallel changing the coating time to 6 h and 2 h. Both samples were also sonicated to test the coating stability.

Table 11: Experiment 8

| **Silanization** | |
|---|---|
| Activation | UV (15min) |
| Silane | TESPSA (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 5 g/L |
| Coating time | 6 h |
| Ultrasound | Yes |

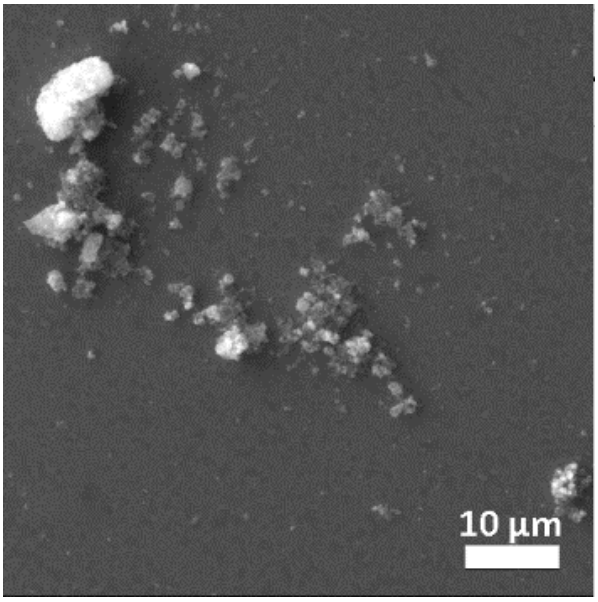



Figure 44: experiment 8 TESPSA x4300

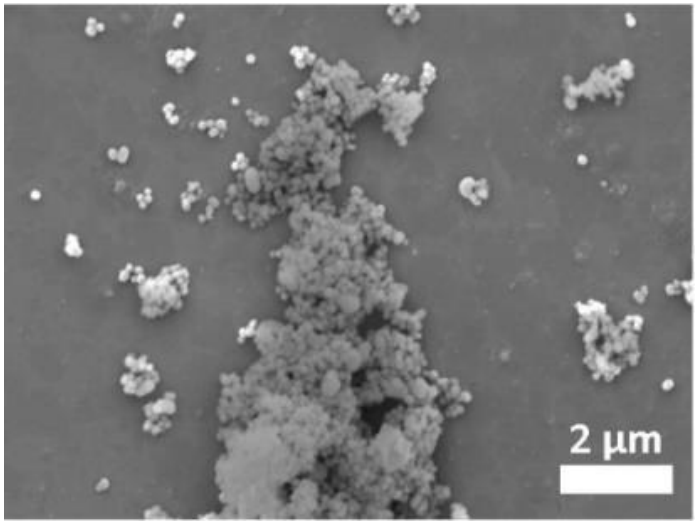



Figure 43: experiment 8 TESPSA x10000

*Table 12: Experiment 9*

| **Silanization** | |
|---|---|
| Activation | UV (15min) |
| Silane | TESPSA (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 5 g/L |
| Coating time | 2 h |
| Ultrasound | Yes |

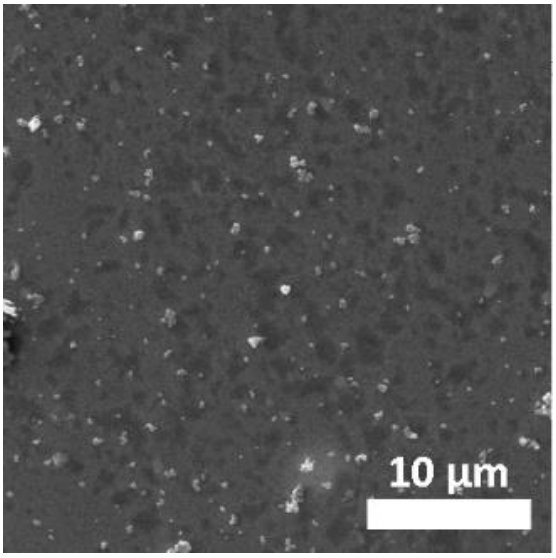



*Figure 45: experiment 9 TESPSA x8000*

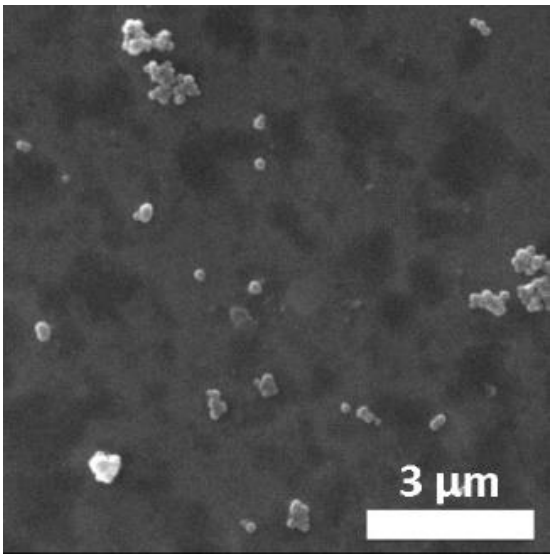



*Figure 46: experiment 9 TESPSA x27000*

For the previous two experiments, the MSN coating was unsatisfactory because only few particles bound the surface. However, a difference between these two results could be shown. Indeed, on the 2-hour experiment image very few cluster were seen, it was mostly nanoparticles well distributed on the surface. On the other 6-hour experiment picture, there were more clusters poorly distributed.

The other strategy to obtain a thin MSN layer on the titanium samples was to decrease the MSN concentration to 2.5 g/L. The next experiments were carried out for two different times, overnight and 6 h:

Table 13: Experiment 10

| **Silanization** | |
|---|---|
| Activation | UV (15min) |
| Silane | TESPSA (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 2.5 g/L |
| Coating time | Overnight |
| Ultrasound | Yes |

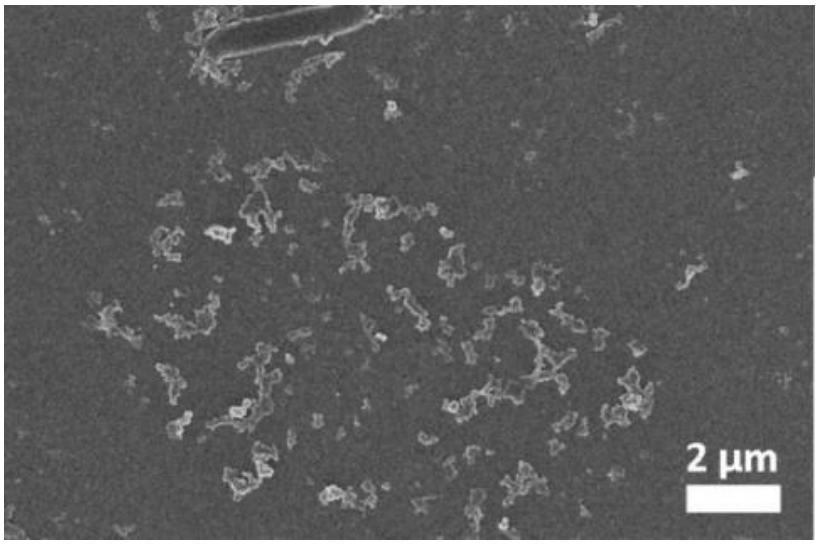



Figure 47: experiment 10 TESPSA x17000

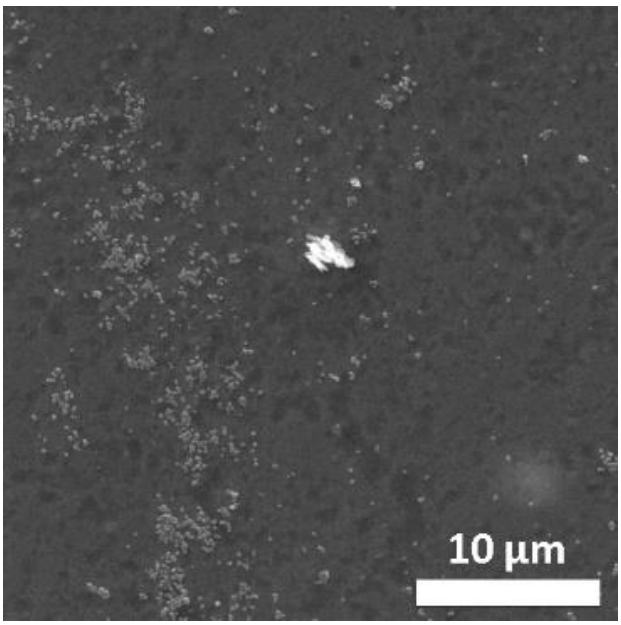



Figure 48: experiment 10 TESPSA x8100

*Table 14: Experiment 11*

| **Silanization** | |
|---|---|
| Activation | UV (15min) |
| Silane | TESPSA (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 2.5 g/L |
| Coating time | 6 h |
| Ultrasound | Yes |

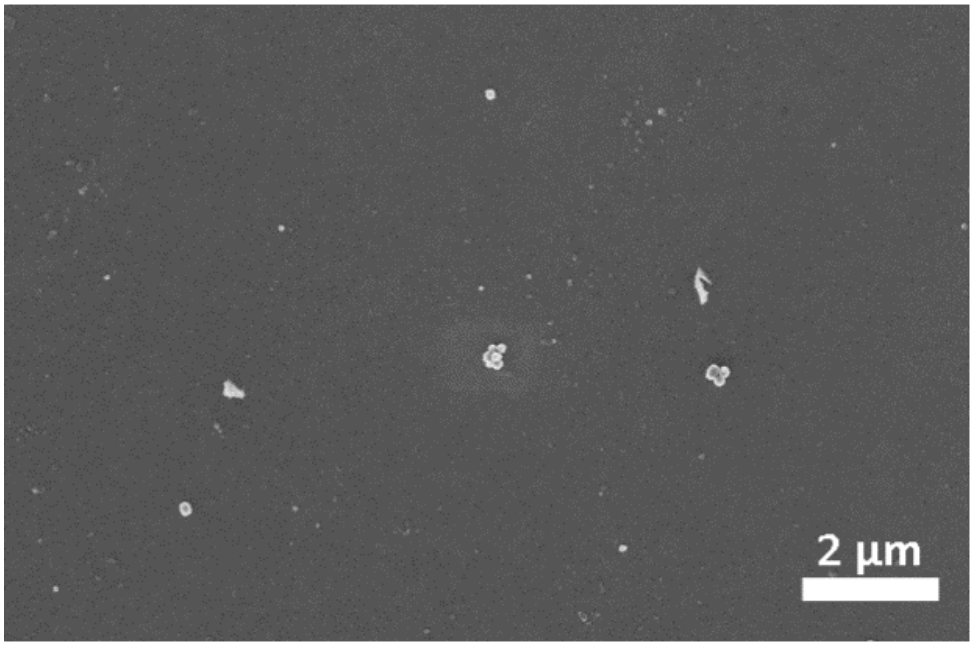



*Figure 49: experiment 11 TESPSA x27000*

SEM results showed that very few MSN and clusters bound the surface, despite the fact that the coating time and the initial MSN concentration were reduced. It seemed that the bonding between the MSN and the surface wasn't entirely covalent, given that ultrasounds wash removed almost all the MSN. Therefore, the changes in the parameters weren't as efficient as expected. The UV activation of the titanium sample could be the origin of the problem.

## NaOH etching activation

To solve this coating problem, two experiments were carried out with a different activation process, in which is necessary to realise a chemical attack of the titanium oxide layer with a basic solution. The objective of this experiments is the formation of more hydroxyl groups at the titanium surface. This method, described in the literature [41]–[43], uses 5M NaOH solution and allowed peptides to be bound on titanium by silanization. As an adaptation of this reported method of attachment, NaOH solution would hypothetically enhance the efficiency of the attachment of MSN.

*Table 15: Experiment 12*

| **Silanization** | |
|---|---|
| Activation | NaOH (24 h, 60 °C) |
| Silane | TESPSA (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 5 g/L |
| Coating time | Overnight |
| Ultrasound | no |

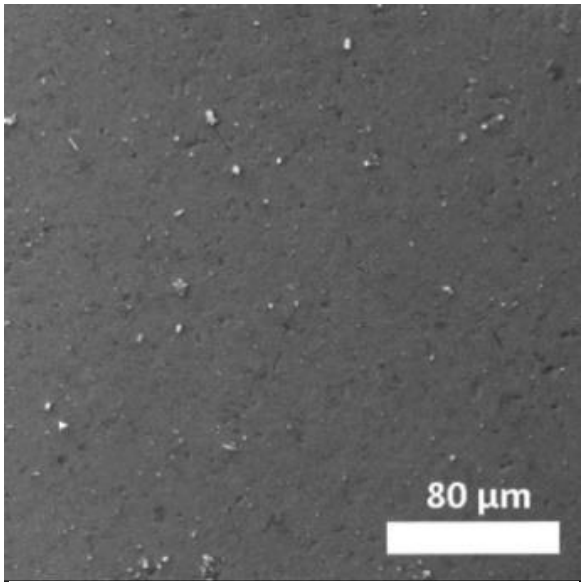



*Figure 51: experiment 12 TESPSA x1000*

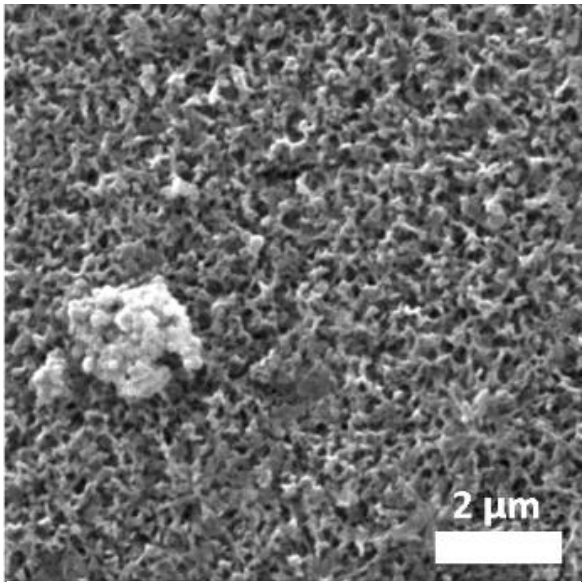



*Figure 50: experiment 12 TESPSA x30000*

| **Silanization** | |
|---|---|
| Activation | NaOH (24 h, 60 °C) |
| Silane | TESPSA (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 5 g/L |
| Coating time | Overnight |
| Ultrasound | Yes |

*Table 16 : Experiment 13*

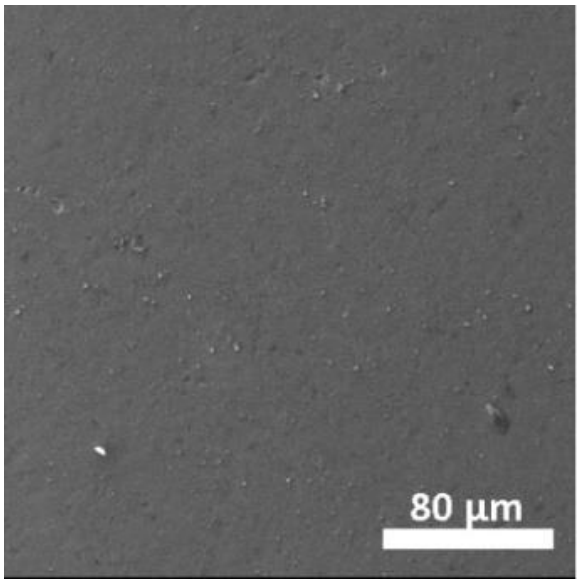



*Figure 53: experiment 13 TESPSA x1000*

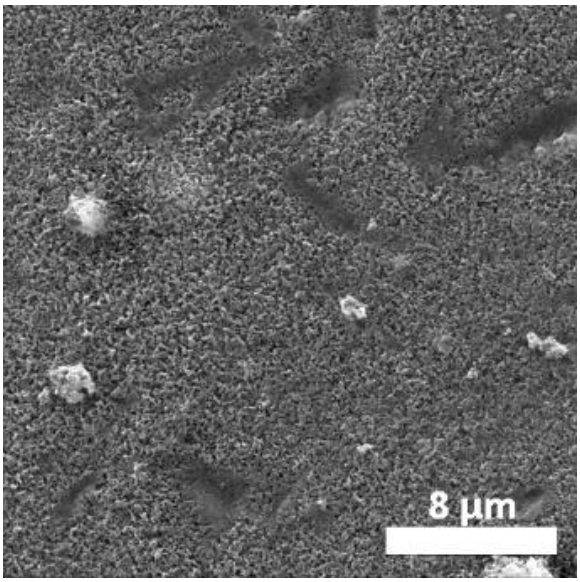



*Figure 52: experiment 13 TESPSA x10000*

As seen on the previous pictures, there were only a few MSN attached to the surface, and the sample sonicated had less clusters than the one manually washed.

The results obtained weren't any better than previous trials. Due to these defects on the coated surface, a new activation procedure was proposed and analysed: the plasma activation.

## $O_2$ plasma activation

The last experiment realised with a new TESPSA as organosilane used dioxygen plasma activation to create hydroxyl groups at the sample surface. This process was previously reported to functionalize titanium surface [44], [45]. Virginia Paredes [46] demonstrated that $O_2$ plasma was more efficient than chemical attack to activate titanium alloys surfaces.

*Table 17 : Experiment 14*

| **Silanization** | |
|---|---|
| Activation | $O_2$ Plasma (10 min) |
| Silane | TESPSA (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 5 g/L |
| Coating time | Overnight |
| Ultrasound | Yes |

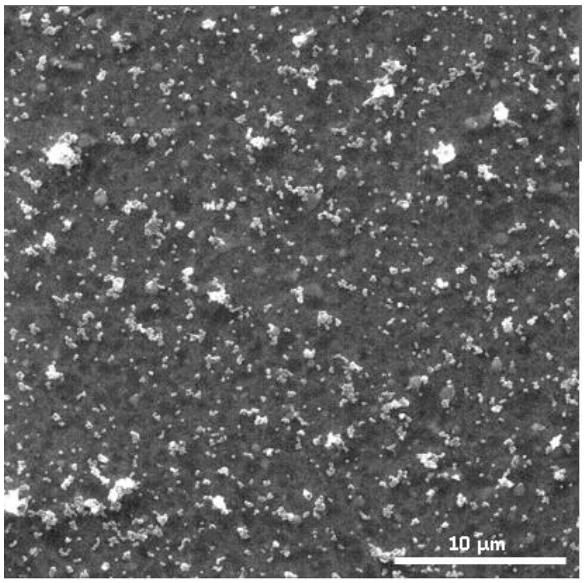



*Figure 55: experiment 14 TESPSA x8000*

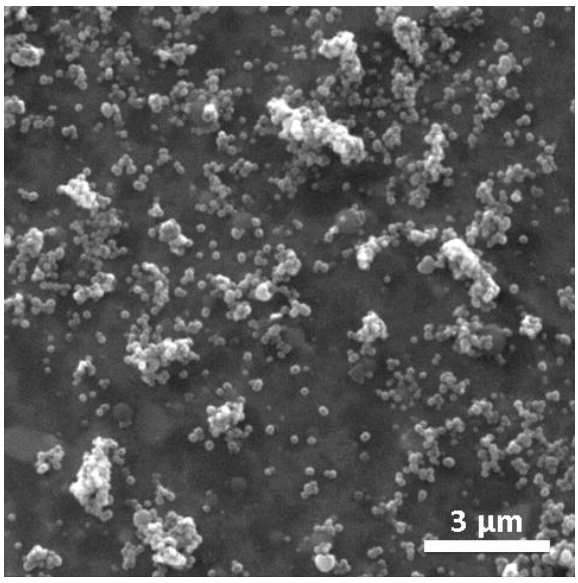



*Figure 54: experiment 14 TESPSA x20000*

*Table 18: Experiment 15*

| **Silanization** | |
|---|---|
| Activation | $O_2$ Plasma (10 min) |
| Silane | TESPSA (2%, v/v) |
| DIEA | 3%, v/v |
| Anhydrous toluene | 10 mL |
| **Coating** | |
| [MSN] | 5 g/L |
| Coating time | Overnight h |
| Ultrasound | No |

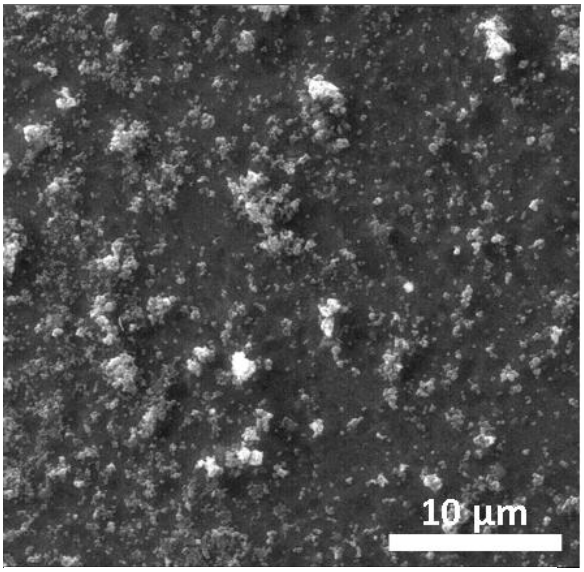



*Figure 57: experiment 15 TESPSA x8000*

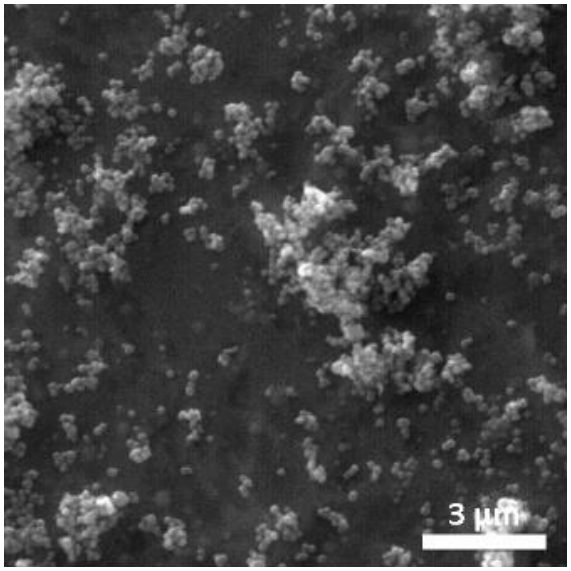



*Figure 56: experiment 15 TESPSA x20000*

The SEM images showed that the MSN were well distributed and attached to titanium. The few remaining MSN clusters were smaller than the previous coatings. At this time, the coating was homogenous, and the amount of MSN was rather acceptable in comparison to the previous experiments. In addition, samples sonicated showed slightly less clusters than manually washed samples. Such coating could be associated to an enhanced activation of titanium. Thus, these improvements would induce better silanization results.

## 4.3. Discussion

Regarding the SEM images, the first objective of depositing a thin layer of mesoporous silica wasn't achieved at the beginning. Indeed, two main cases were seen: either some samples weren't coated at all (or very few MSN bound to the titanium), or the sampled showed a coating with too much material. These results could be explained by some hypothetical reasons.

The first coating layers using ICPTES were too thick. At this point, the process excluded the final ultrasound washing step after the coating. Hence, it wasn't possible to tell if the layer observed was attached with strong covalent interactions.

The next experiments were executed more carefully, and the manual washing step was compared with the ultrasound washing process. Applying these conditions, much less MSN clusters were observed on the sonicated titanium substrate. However, this time the material amount was too low. The samples showed either few MSN disseminated or some clusters poorly distributed.

To explain this sudden lack of material adhesion, the process was reviewed to fix the coating problems. First and foremost, the surface activation step could be not as effective as expected. Thus, less hydroxy groups would have been formed at the titanium surface, which induces a less effective silanization. This could be the case of UV and NaOH activation. The dioxygen plasma activation seemed to activate the whole surface homogeneously.

The silanization reaction used three different reactants which are relatively sensitive to humidity. These chemicals could have been degraded by hydrolysis. Indeed, the ICPTES wasn't used with septums to prevent any contamination at the beginning of the study. The hydrolysis reaction could also occur during the silanization process: the eventually be caused by a poor dinitrogen purge or a poorly washed reactor. Also, a ninhydrin test was executed to check the ICPTES quality. As the test was positive, new ICPTES was used, and TESPSA was studied.

Also, the polycondensation of the silane precursors could also be an obstacle to the thin MSN layer formation. Indeed, the perfect conditions for the thin layer to form would be a polycondensation that would follow the plane geometry of the titanium surface. However, this is not necessarily the case, the polycondensation can actually keep going upwards. Thus, it could explain the non-homogeneity of the layer at the titanium substrate surface.

Another idea of what could have affected the coating yield is the MSN quality. To obtain the covalent bonding between the silanized titanium substrate and the particles, both parts need to have their own reactive groups at the surface. The succinic anhydride or the isocyanate groups of the silanized substrate, and the amine groups at the surface of the nanoparticles. It is possible that the amine groups concentration at the surface of the nanoparticles could be insufficient to be properly attached to the silanized substrate.

The results of the last ICPTES and TESPSA experiments with plasma activation were the more convincing, given that the material amount on the silanized sample was rather acceptable, and the MSN were well distributed. Thus, the conditions of these experiments should be kept for further process optimization, especially for the plasma activation, the silane precursor and the sonication step of the coated samples. Indeed, the plasma activation is a simple and rather quick method to activate the titanium surface. Regarding the two silane precursors, it seems that the TESPSA is more convenient to handle and should be preferred for the silanization.

However, the silanization and the coating process still need to be enhanced. The parameters to be tested for further research would be the coating time and concentration, and optionally the proportion of silanization reactants (TESPSA/DIEA/anhydrous toluene).

# 5. Conclusion

This project gave the opportunity to develop a new composite material and to study the reaction of silanization used as a coating process for titanium substrate. Many experiments were carried out in order to determine which conditions are the most appropriate to get a thin coating, in anticipation of further loading and release studies.

Over all these experiments, the MSN coating was at first difficult to obtain. The sensibility of the chemicals and the numerous parameters during the silanization and the coating procedure made the experiments rather delicate to realise. Moreover, the MSN synthesis was also a key step for the whole process. The loading and release study could not be done, however, these experimental results finally led to a MSN coating which potentially could be loaded with drug.

Regarding the last experiments, plasma activation techniques should be kept, for its simplicity, rapidity and efficiency, even though the UV activation process was certainly less expansive. The NaOH activation could be also studied, but it cannot really be compared to the other processes, since the surface roughness obtained is completely different.

According to the SEM images, the TESPSA was more stable than the ICPTES. If such silanization and coating steps are mastered, other characterization procedures like FTIR in ATR mode, should be applied to ensure that the bonds between the silanized titanium and the MSN are covalent.

In a parallel project, loading and release of nifedipine in MSN have been studied. A method of loading has been optimized and could be used as basis to load nifedipine in MSN coating on titanium. Applied to coated titanium substrates, this method would consist in the loading of nifedipine in methanol as solvent using a rotary evaporator in which the plate would be placed.

Thus, to go further with this project, loading and release study should be done. It may be easier to go on with TESPSA as the only silane precursor to simplify the study, since it was less sensitive. A first loading study by ATR FTIR characterization for instance, could give a first idea of the drug carrying performance of such composite material.

In addition, it would be useful to keep on optimizing the silanization process, in other words, to increase either the coating time or the MSN solution concentration to see if the titanium could stand a more important coating that remains stable. Then, such coating could be loaded as well and compared to the former. The best coating could then be used for the release studies.

To sum up, the best coating conditions obtained so far were:

| Activation | Organosilane | MSN concentration in solution | Coating time |
|---|---|---|---|
| **$O_2$ Plasma (10min)** | **TESPSA** | **5 g.L$^{-1}$** | **Overnight experiment** |

# Economic impact

An economic analysis of the project has been done to give an approximation of the overall cost of this project. This analysis considers all the costs related to the different step of the project. This includes the raw materials, the chemicals, the various consumable tools, the personal (PhD student, tutors, technician) requested, and the high-technology equipment.

The total cost of the project would approach approximately 11 265.72 €.

*Preparation of samples*

| Resources | Quantity | Price | Cost (€) |
|---|---|---|---|
| Titanium bar (Ø10 mm) | 0.2 m | 368.45 €/m | 73.69 |
| Bakelite embedment device | 7 h | 10 €/h | 70 |
| Automatic polishing device | 24h | 30 €/h | 720 |
| Grind paper 305 mm P600 | 6 u | 150 €/100 | 9 |
| Grind paper 305 mm P800 | 12 u | 150 €/100 | 18 |
| Grind paper 305 mm P1200 | 12 u | 100 €/100 | 12 |
| Grind paper 305 mm P2500 | 12 u | 450 €/100 | 56 |
| Polishing 305 mm | 4 u | 37.82 €/u | 151.28 |
| Colloidal alumina 1µm | 2 L | 60 €/L | 120 |
| Colloidal alumina 0.05µm | 2 L | 60 €/L | 120 |
| Total | | | 1349.97 euros |

*Cleaning solvents*

| Resources | Quantity | Price | Cost (€) |
|---|---|---|---|
| Distilled water | 1L | 0.08€/L | 0.08 |
| Acetone | 1L | 16.65€/L | 16.65 |
| Ethanol | 1L | 25.5€/L | 25.5 |
| Cyclohexane | 1L | 37.21€/L | 37.21 |
| Total | | | 79.44 euros |

*Silanization and coating*

| Resources | Quantity | Price | Cost (€) |
|---|---|---|---|
| NaOH | 4 g | 16.30 €/kg | 0.0652 |
| Anhydrous toluene | 500 mL | 47.80 €/100mL | 239 |
| DIEA | 4.8 mL | 42.64 €/100mL | 2.04672 |
| TESPSA | 3.2 mL | 39.4 €/100mL | 1.2608 |
| ICPTES | 3.2 mL | 39.4 €/100mL | 1.2608 |
| Total | | | 243.63 euros |

*MSN synthesis*

| Resources | Quantity | Price | Cost (€) |
|---|---|---|---|
| NaOH | 4 g | 16.30 €/kg | 0.0652 |
| CTAB | 2 g | 302.1 €/g | 603.2 |
| TEOS | 9 mL | 47.80 €/100mL | 239 |
| APTES | 2 mL | 42.64 €/100mL | 2.04672 |
| Deionized water | 1L | 39.4 €/100mL | 1.2608 |
| Methanol | 450 mL | 39.4 €/100mL | 1.2608 |
| Ammonium nitrate | 4 g | 87.5 €/kg | 0.35 |

Total 847.26 euros

*Laboratory staff and high-technology*

| Staff | Hours | Price | Cost (€) |
|---|---|---|---|
| SEM | 32 | 40 €/h | 1280 |
| FTIR | 3 | 5.14 €/h | 15.42 |
| Director | 60 | 60 €/h | 3600 |
| PhD student | 120 | 30 €/h | 3600 |
| Project student | 400 | 0€/h | 0 |
| Others (tubes, boxes, gloves, etc.) | | | 250 |

Total 8745.42 euros

# Environmental impact

In addition of safety instructions and preventive measures, which are essential to avoid any accident, the BBT laboratory group is following a set of guidelines in order to reduce the environmental impact at several levels. Indeed, the laboratory environment, apart from being potentially dangerous, is often releasing toxic substances, which can be gases, solids or liquids. These same chemicals are likely polluting substances that could cause severe damages to the environment.

Therefore, every single person working in the laboratories must be fully aware of these environmental risks and must follow specific procedures. Every experiment should use the least amount of material possible to not waste it. Also, it is very important to know where each chemical must be thrown. Indeed, each type of chemicals has its dedicated bin. The same applies for the empty bottles which are recovered and kept in a closet. These special chemical residues are taken up by a specialized company, which treat them properly.

Thus, by such systems and raising awareness of the environmental impacts by the laboratory groups, the ecological damages are limited.

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